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ETIOLOGY OF TSUTSUGAMUSHI DISEASE*

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An endemic infectious disease, called Tsutsugamushi disease (Kedani fever, river fever), occurs in Japan along the rivers in the northern provinces. In spite of the persistent efforts of numerous workers, its etiology has been very difficult to determine. This peculiar disease has a close resemblance to Rocky Mountain spotted fever in America, but it is transmitted by a different insect. The carrier of the Japanese disease is a minute red mite, abundant in the infested region, that is, in the low lying cultivated fields along the Shinano river, between the riverside and embankments which vary up to one and a half miles from the river. These fields are at times during the summer covered by overflow water. In nature the mite is ectoparasitic in the ear of field mice, but it freely attacks human beings as well as other animals whenever accessible. Zoölogically, it is considered *Leptus akamushi* (Brumpt), a species distinct from the well known *L. autumnalis* of Europe.

The early clinical course of the disease is marked by a gradual rise of the body temperature, a characteristic lesion at the site of the bite, and swelling of adjacent lymph nodes. The incubation period is about eight days. The fever, which is usually 38°-39° C. in the early stage, may rise to 40° C. or even higher, and usually lasts three or four weeks. Complete recovery requires a month or more. In bad cases a fatal result follows about ten days after the onset. The mortality of the disease is approximately forty per cent.

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*My work on this disease was started in 1906 and has been continued up to 1919. Many of my findings in the course of these twelve years' investigation have been published in Japanese medical journals (Hokuetsu Igakkai Zasshi, no. 156, 1906; no. 158, 1907; no. 162, 1908; no. 165, 1909; no. 168, 1909; no. 173, 1910. Chu-o Igakkai Zasshi, no. 124, 1915; no. 127, 1916; no. 130, 1916. Hayashi, N., Mukoyama, T., Oshima, F.: Chu-o Igakkai Zasshi, no. 138, 1918; 1919 [xxvi] no. 2). A summary of these findings together with new facts and conclusions with regard to the etiology of this disease is of especial interest in connection with recent American contributions to the study of Rocky Mountain spotted fever. I have been very much pleased to find in Dr. Wolbach's recent detailed description of the organism found in the American fever, a very close similarity to the bodies which I found in the closely related Japanese disease.

The microscopical examination of fresh material in the hanging drop shows a number of refractile, spherical bodies, each measuring from 1 to 3μ in diameter, without any perceptible movement. They rarely occur in clusters, but two or three of them may often be seen linked together. As a rule, when two bodies are coupled, one is decidedly larger than the other. The smaller half of the couple often appears as if it were a pseudopodium of the larger. I discovered these peculiar bodies in 1906, and designated them as the Spheroid Bodies. The examination of blood shows, in addition to these, others which I have called the "rod-bodies." There are two kinds, the first large and the second very small; the large one measures about 7 by 3μ. It contains a spherical refractile area resembling a nucleus and a number of much more refractile granules toward the periphery, and is always found close to the surface of the red blood cells. The small "rod body" measures about 3 by 0.5μ and resembles a bacterium. It shows no evidence of motility.

Varying numbers of the spheroid as well as rod bodies are often found embedded in the cytoplasm of the cells of the exudate and of lymph nodes. Fresh sections examined in normal salt solution, glycerine or dilute acetic acid show these bodies intracellularly; and their morphological characters are identical with those that are found extracellularly.

The lesion which develops at the location of the bite is quite unique, being comparable only with the chancre of syphilis. In the earliest stage that can be identified, the wound is much raised and is about the size of a small pea, being 2-3 mm. in diameter. It has first a reddish purple color, and later a blackish necrotic appearance. A smear from the wound at this stage shows a large number of very small rod and spheroid bodies, but cultures taken from the same source at the same time are entirely negative. The wound usually commences to heal through the regeneration of surrounding tissues when the case is on the way to recovery, and thus shows close parallelism with the general clinical course of the disease. Obvious as is the importance of the study of the wound, it has received little attention. On account of its being exposed, thus offering opportunity for various secondary infections, the result of observations are of little value unless controlled by bacterial cultures in every case.

At the onset of the fever, the minute bodies appear in great abundance in the smear. Most of them are seen to be more or less enlarged. Even at this stage cultures do not demonstrate any bacterial infection. At the height of the fever, when the superficial, degenerated tissue of the lesions falls off, leaving a small ulcer, there usually occurs a secondary infection with common bacteria such as staphylococci, as

detected by cultures. In mild cases, the rod and spheroid bodies, as well as bacteria, gradually decrease in number, hand in hand with the decline in fever.

In later stages, with necrosis and ulceration of the lesion, the exudate usually contains bacteria. The wandering cells which appear in large number in the lesion are actively phagocytic. Some of these phagocytes contain in addition to degenerative products of red blood cells and tissue cells, a large number of rod as well as spheroid bodies. The manner in which the minute bodies are included in cytoplasm of these cells is identical with the conditions in the cells of lymph nodes.

The rod-bodies are extremely small, measuring 1-2, rarely 3μ in length. Loeffler's methylene blue and ordinary Giemsa solution stain them blue throughout like bacteria, but Prowazek's trachoma granule stain brings out in the rod-body, a circular area of chromatin substance staining purplish, leaving the rest of the body blue although not as distinct as in protozoa. Hematoxylin also gives a staining reaction of the circular area characteristic of chromatin. The rod-body is typically more or less pointed at one end, and slightly rounded at the other; the chromatin area being at the round end (Pl. X, Figs. 18, 24). Some of the rod-bodies show a chromatin area at each end (Figs. 19, 20, 21). In those that are as long as 3μ the chromatin substance may appear to lie outside the body and attached to one end of the latter by a narrow belt of achromatic substance. (Pl. X, figs. 25, 26; Pl. XI, figs. 3, 5). The largest of the rod-bodies may measure 7 by 2μ and may even at a glance appear like trypanosomes (Figs. 15, 16). These giant forms also contain either at the end or middle, one or two masses of chromatic substance.

These spheroid bodies are comparatively large, being 2-3μ in diameter, and stain deeply like cocci. A characteristic figure often seen shows two spheroid bodies of distinctly different sizes coupled together (Pl. X, Figs. 27, 28, 31), or in a manner resembling diplococci (Fig. 29, 30, 6 B), or even in chain formation. Frequently chromatic substance in the center stains more deeply than the peripheral area. These characteristic features are more conspicuous in larger forms than in smaller ones.

The swelling of lymph nodes adjacent to the bite takes place in the early clinical stage, when the wound is still elevated and the body temperature only slightly raised. Although some lymph nodes were removed by operation in an early stage the greater part of my material was obtained after the development of distinct fever (38.5°-39° C.). All of the lymph nodes in the region manifest swelling though in different degree. At the height of the fever, material was often taken by puncture with the syringe; it was collected at autopsy, and also by operation at various stages in experimental infection of animals.

Examination of sections and smear preparations has shown the presence of peculiar granules, which are taken up by the giant phagocytes of endothelial nature. These granules and phagocytes occur most abundantly around the bite in the adjacent lymph nodes and spleen. Morphologically, three types of granules and phagocytes are found in the cytoplasm of the large mononuclear lymphoid cell, namely:

(a) A large ring-shaped chromatic body with an achromatic area (Pl. I, 1a; Pl. XI, Figs. 10a, 11a, 12a, 13a).

(b) A spherical body showing deep and uniform blue staining, but containing reddish chromatin substance having a bipolar distribution, and resembling a diplococcus (Pl. IX, Fig. 1B, 1b).

(c) An exceedingly small rod shaped body, often more of comma-shape, or with dumb-bell-like construction. This third type of the granules is most abundant (Pl. IX, Figs. 2, 3, 4, 5).

In 1915, I found granules identical with those just described in small lymphocytes from lymph nodes removed early by operation. Examined with the Giemsa-Prowazek stain, minute granules can be demonstrated in the narrow area of blue cytoplasm around the purple nucleus. With the aid of a Zeiss $\frac{1}{12}$ objective and ocular 6 or 8, the granules can be easily classified into the three types already described. Here, more than in other cases, the rod-shaped type predominated. The rod-body is more or less rounded at one end, and pointed at the other. A proper stain shows the round end reddish purple, and the pointed end blue. Delafield's hematoxylin brings out similarly the three types of granules, and also differentiates clearly the round end of the rod-body.

In general, the granules are fairly constant in size. The number embedded in a single cell varies from a few to approximately two hundred, but more commonly from ten to sixty. These are usually more or less localized in the enlarged portion of the cytoplasm (Pl. IX, Fig. 2; Pl. XI, Figs. 2a, 3a), or in the area of the cytoplasm formed by the indented nucleus.

The lymph (from lymph nodes) also contains three types of the granules. Here, too, the granules may be found in clusters in many cases, although some may be solitary. In addition, the fluid often contains a fourth type of granule, round in shape with a centrally located red-staining spot and a narrow achromatic ring, which is in turn surrounded by a finely granular peripheral zone (Pl. XI, Figs. 17, 18, 19). I have given the name of "oocystoid body" to this type.

It is natural to suppose that the virus of Tsutsugamushi disease may be found in the blood, after it enters the body of the patient through the bite. Its demonstration would be of great value, especially as affording a comparatively simple means of accurate diagnosis. Cover

glass smears from a large number of severe cases all showed the presence of the peculiar granular bodies, in or on the red corpuscles. In mild cases, or those in very early or late stages, none, or a very small number of the bodies were observed. These bodies may also occur in any type of white blood cell, and, moreover, may be seen scattered freely in the blood plasma. These facts I reported in 1906.

The granular bodies are minute, strongly refractile, and usually found within or on red corpuscles (Pl. X, Figs. 6c, 7). A highly magnified picture of Giemsa-Prowazek stained specimens (Pl. X, Figs. 6, 7) shows that these bodies are in no way different, as far as could be observed, from the minute bodies already described in the lymphoid cells. They are usually comma-shaped, but often modified into rod or dumb-bell shapes. They are, as a rule, evenly distributed within the cell, although sometimes grouped in one part of it. The number of the bodies in a red cell varies from a few to many. Enlarged forms, such as are found in the cells of the lymph nodes, also occur in the blood (Pl. X, Fig. 6A, 14). The minute bodies may be seen free in the blood plasma during the eruptive stage even in the case of a slight attack. The smaller forms of these extra-cellular bodies may appear in a thickly crowded group (Pl. XI, Fig. 1, 16a), while some of the larger forms may be found coupled with smaller ones (Pl. X, Figs. 15, 16, 17). This phenomenon of coupling among the larger bodies is of special interest, as it takes place only in very severe cases.

Sometimes with the Giemsa-Prowazek stain a chromatin mass surrounded by an achromatic ring was seen at one end of the body (Pl. X, Figs. 12, 13, 14). Some bodies are round (Figs. 12 13), others elongated (Fig. 14). The former is like a ring body of the malaria plasmodium; the latter has two (end and center) chromatin spots, the central being very small. Delafield's hematoxylin also brings out these chromatin spots.

The enlargement of the spleen in Tsutsugamushi disease, usually encountered at autopsy, can be easily detected clinically. Material taken by puncture with a syringe always contains a large quantity of red cells, but pulp-cells are rarely seen. The minute granular bodies are abundantly demonstrated in these cells in many cases. This is also true in the case of autopsy material. Generally speaking, the smaller type of granular bodies seems to predominate in this organ (Pl. XI, Fig. 8a).

ANIMAL EXPERIMENTS

1. Infection through the bite of the mite: Animals (monkeys, rabbits, guinea-pigs, rats and calves) were allowed to be attacked by the mites in the infested region, and after recognizing the evidence of the bite on them, development of symptoms was carefully watched for.

The monkey shows the most typical rise of body temperature, the guinea-pig a less typical fever, and the calf only a slight rise of temperature. The rabbit shows no change.

Positive infection of a monkey (*Pithecus fuscatus* Blyth, the short-tailed species occurring in Thikoku, Japan) through the bite of the mite was first proved by the author in 1906. In this animal, the typical lesion of the bite developed, along with the enlargement of the lymph nodes and the characteristic fever, four or five days after the bite was recognized. The general clinical changes closely resemble those in human cases, even to the occurrence of leukopenia. The characteristic minute bodies, described from human cases, are present in the phagocytic cells of lymph nodes and spleen; in a later stage, also in those of bone marrow, and still later in most of the other internal organs. These bodies were also found free in body fluids. The infected monkeys usually recover in a week or two.

Guinea-pigs show no special pathologic change at the site of the bite, although swelling of adjacent lymph nodes and fever develop within several days after they are bitten. In these cases, too, the minute bodies of several types were abundantly demonstrated in the lymph nodes, blood and other tissues.

2. Transmission Through Injection of Infected Blood: The typical symptoms of the disease were successfully reproduced in the monkey by subcutaneous injections of the blood from the severe human cases. The diseases can also be transmitted from the monkey thus infected to other monkeys by inoculating them with blood drawn from the former. Similar experiments with guinea-pigs also gave positive results. Moreover, the injection of blood or emulsified organs from infected guinea-pigs into other animals, into monkeys, was followed by the development of unmistakable symptoms.

3. The Relation of the Mite and Field Mice: As stated already, the mite is parasitic on the inside of the external ear of the field mouse, which is very abundant in the infested region. In 1910, I was able to demonstrate that the field mouse is a bearer of the virus of Tsutsugamushi disease.

Aerobic and anaerobic cultures, on various solid as well as liquid media, were repeatedly taken from a large number of human and animal cases, always with negative results. A few of the common varieties of bacteria or often yeasts developed in some cultures, but no organism that can be considered as the causative agent of the disease has ever been detected in any of them.

By using Kleine's (1905) piroplasm medium, it was possible to recognize the presence of the minute bodies described in this paper, but no definite growth has been demonstrated on this or on the Novy-Mac-

Neal-Nicoll medium. The cocci bodies, grown on Loeffler's serum-agar by Nagayo and his associates in 1917, could not be found in repeated experiments which I made with the same medium. The possibility of cultivating the virus of the disease has not thus far been definitely demonstrated.

Under natural conditions healthy individuals do not contract the infection directly from patients suffering from the disease, the virus being transmitted only through the mite. It has been noted in earlier publications that a certain degree of immunity follows recovery from this disease. A second infection may occur some years later but the case is never severe. A third, or even fourth infection, in a very light form is also known. In animals, however, I found that no second infection was possible, in spite of many trials. I have also noted that the first infection is very mild in children or in young individuals, while in adults over forty it is frequently fatal.

The resistance of the virus to thermal change and putrefaction is very slight, as virus from human as well as animal cases is no longer virulent a day or two after it has been taken. Another important fact is that the virus is absent from the filtrate through Pukall or Berkefeld filters. This point, first reported by Kitajima and Miyajima, was recently confirmed by Nagayo.

DISCUSSION

1. The Granular Bodies in the Lymphoid Cells: The larger types of these ring or spheroid bodies are found in large mononuclear cells, as well as in the cells of the spleen pulp, while the minute type (rod bodies) are embedded in the cytoplasm of small mononuclear lymphoid cells.

Since Ehrlich's classical work it has been believed until very recently that the mononuclear lymphoid cell is not granulated under either normal or pathological conditions. Among others, Pappenheim and Schridde have shown the occurrence of fuchsinophile granules, which are now known to be the true mitochondria. More recently azure granules of problematic nature have also been described in this type of cells. These granules are, however, of different chemical nature from my granular bodies, as can be seen from their staining reactions. It is beyond doubt that the former could not be considered identical, or even genetically related to the latter. An extensive examination has failed to demonstrate the granular bodies in lymphoid cells in normal individuals, leading to the conclusion that they are not normal constituents of these cells. Nor have the granular bodies been observed in the lymphoid cells in any case of lymphoid hyperplasia that I have examined.

The impossibility of the granular bodies being any sort of degeneration products, which might become phagocyted by lymphoid cells, is none the less evident in view of my microscopical observations. The morphological characters and the regularity in the modification of these bodies, as detailed early in this paper, preclude any conclusion that they are degenerative debris, and suggest very strongly their being peculiar micro-organisms. For the same reasons the granular bodies must be considered as different from Prowazek's so-called "reaction products" in the infections of various sorts of filtrable virus. The granular bodies in question are absolutely peculiar to Tsutsugamushi disease and must not be overlooked in the study of its etiology.

2. The Granular Bodies in the Large Mononuclear Cell: The large mononuclear cell, called endothelial in my report in 1916, Kiyono terms histiocyte. The occurrence of the granular bodies in this type of cells is a common finding in Tsutsugamushi disease. The granules are either rod-shaped, spherical or ring-shaped, and these three types may be found side by side in a single cell. They are identical in staining reactions but differ in size. It is very probable that the three types represent stages in a series of modifications undergone by the same organism. It seems that the minute rod-shaped body gradually grows, assumes a spherical form and then after further growth the ring shape. The semilunar, or crescent shape often taken by some much enlarged bodies may be considered as due to still further growth. Some of the ring-shaped bodies may also be coupled. It is very interesting to find on close examination that these enlarged bodies contain a number of exceedingly minute granules. If the interpretation suggested proves to be correct, the granular body must be an organism, and more specifically a protozoon, which has intracellular stages in its life cycle.

3. Granular Bodies in the Red Cell: In view of the fact that these bodies resemble the ordinary basophilic granules in the red cells, the distinctive characteristic between them may be pointed out. As first made known by M. Askanazy, Grawitz and others, the basophilic granules of red cells appearing under certain pathologic conditions, are nothing more than ill-defined, minute particles. Contrary to this the "granular bodies" found by me are large enough to be measured. Moreover, they are very clearly differentiated from the protoplasm of the red cells. On destaining the true basic granules become obscure very rapidly, while the "granular bodies" stand out clearly by their peculiar refractility, sharply defined edges, and finally by the characteristic chromatin spots which still retain a reddish purple color.

In short, the "granular bodies" with their organized structures cannot be confused with the true basic granules which have no definite

morphological characteristics. I conclude, therefore, that the "granular bodies" in the red blood cell are of the same nature as those in the lymphoid cells. Since the red cells as well as the lymphoid cells contain the "granular bodies" only in cases of Tsutsugamushi disease, these bodies must be considered seriously in connection with the etiology of the disease.

4. Free Bodies in Plasma: In the foregoing I have stated that the minute bodies usually found embedded in the cytoplasm of lymph cells may also occur free in the lymph, either solitary or in groups, and that these bodies may be rod, spheroid, or ring-shaped. An oocyst-like appearance was also assumed by some of them. Swarms of the minute "comma" or "rod-bodies" often mixed with longer dumb-bell shaped ones, were observed in the plasma in blood smears. Also these bodies have been found in addition to bacteria in serum from the wound at the site of the bite.

The free bodies are not abundant in mild cases but in severe cases their appearance is conspicuous. The free bodies often appear earlier than the intracellular bodies, and may occur in the wound along with ordinary bacteria.

Since the minute bodies in lymphoid cells are peculiar to Tsutsugamushi disease, the free bodies should also be considered of the same significance inasmuch as they have not been found under any other conditions. Morphologically, moreover, a minute comparison fails to detect any perceptible difference between the intracellular and free forms.

In their staining reactions and morphology alone the bodies which I have described often resemble bacteria, especially as the cytoplasm and chromatin substance are differentiated with difficulty, but there is no other evidence of their bacterial nature. In smears from blood and lymph nodes I have found bodies in both red cells and lymphocytes identical in appearance with the ring forms found in the red cells in malaria (Pl. IX, Fig. 1a; Pl. XI, Figs. 4b, 5b), showing a structure never found in bacteria.

I described these minute granular bodies in my first report on the virus of Tsutsugamushi disease and from various findings concluded that they represent a species of protozoa. It was suggested that the organism might be either Piroplasma or some related form. In a further paper I classified the organism as Piroplasma, referring to *Theileria parva* of African cattle fever, and tropical pirosomes in cattle described by Dschunkowsky and Ruhs, as most closely related forms.

In order to indicate the basis of my conclusion, the following facts should be emphasized :

1. Tsutsugamushi disease is transmitted by certain species of mite; the original bearer of virus in the infected region is the field mouse on which the mite is parasitic.

2. Tsutsugamushi disease is characterized clinically by the lesion, high fever, and swelling of lymph nodes. Decrease in the number of leukocytes is well marked in the blood picture. There is also a gradual decrease in red cells, but it is not conspicuous.

3. Anatomically the lesion is characteristic, also the swelling of lymph glands and splenic enlargement; the blood is less coagulable. Internal organs undergo parenchymatous degeneration and often show small areas of necrosis and thrombi.

4. Experimentally it is possible to transmit the infection with typical symptoms by inoculation of fresh blood or other tissues, from human as well as animal cases experimentally.

5. No bacteria which can be considered as the cause of the disease have been cultivated on media, either from clinical or experimental material, nor have any spirochaetes been found.

6. Under normal conditions the virus is not transmitted directly from man to man. It is least resistant to dryness and heat, and unfilterable.

With the above facts on one hand, and on the other the knowledge that the rod, spheroid and ring-shaped bodies, occur in all internal organs and in the blood and especially because of the early appearance of the rod-bodies in lymph cells adjacent to the bite, I have reached the conclusion that the virus of the disease is the species of Piroplasma in question. Among various species of Piroplasma, the cause of African cattle fever, *Thieleria parva,* (see Gonder, 1911), seems closely allied to the forms found in Tsutsugamushi disease, on account of morphological similarity, and of affinities for lymphocytes or endothelial cells.

In the case of *Theileria parva,* however, it is impossible to infect normal cattle by subcutaneous injection of diseased blood, while in Tsutsugamushi disease such a transmission is easily secured through an injection of a very small quantity of infected blood. In this respect the tropical pirosomes, described by Dschunkowsky and Ruhs (1904) from the Caucasus, shows a closer approach to my organism since in the former infection can be transmitted mechanically, as in the latter. In addition the tropical pirosome agrees with my granular bodies of Tsutsugamushi disease in having an intracellular stage and also three different forms, rod, spheroid and ring bodies.

The tropical pirosome deviates from the organism found in Tsutsugamushi disease in its typical protozoon staining reaction, and also in

that it does not infect man and cannot be transmitted experimentally to monkeys. The blood parasite of the mole, *Grahamella protista,* Wolbach's (1914) organism in Rocky Mountain tick fever, and *Bartonia bacilliformis* described by Strong and others (1915) in oroya fever, are also more or less closely related but are undoubtedly specifically distinct. I consider the organism in Tsutsugamushi disease as a hitherto undescribed species, and at the suggestion of Dr. Henry B. Ward designate it as *Theileria tsutsugamushi,* n. sp. I am inclined to believe that further study will justify the inclusion of this species in a new genus clearly distinct from that in which it is placed here.

THE PROBABLE LIFE CYCLE OF THE ORGANISM

Since it has not been possible to cultivate the organism, the following successive changes in preparations is the only method for studying its life cycle. For this purpose, all the varieties of granular bodies I have observed were faithfully sketched from preparations (Plate XI):

(a) In Lymphoid Cells: The granular bodies in their smallest form (rod-shaped) are at first chiefly localized in one part of the cytoplasm (Figs. 2a, 3a, 4a). As they increase in size, they become more evenly distributed in the cell body (Figs. 5a, 6a, 7a, 8a) and some show dumb-bell, spheroid, or even ring-shapes. Cells containing large bodies, (Figs. 9a, 10a, 11a, 12a, 13a) gradually disintegrate, setting these bodies free (Figs. 14a, 15a, 16a). At the same time, the chromatin portion of the larger body becomes granular in structure and finally breaks up into minute comma-shaped granules (Fig. 1).

(b) In Red Cells: The changes are similar. A group of minute granular bodies (Figs. 1b) gradually breaks up as they grow into enlarged rods (Figs. 2b, 3b) which continue to increase in size (Fig. 4b) until they become ring-shaped and finally dissociate themselves into minute granular bodies.

(c) In the Plasma: Arranging the free granular bodies according to sizes one would naturally take the minute granular form (Fig. 1) as the starting point, successively followed by the comma or rod form (Figs. 2, 3) and by the spheroid (Figs. 4, 5, 6, 7, 10) or ring-shaped, malaria-like form (Figs. 4 to 9). They may then assume an amoeboid appearance (Figs. 11 to 17) and elaborate minute granules within themselves. These may early break up into minute comma-shaped bodies (Fig. 1) or may form Koch's free "Plasmakugeln" (Figs. 19 to 22) and finally dissolve into comma or rod bodies (Fig. 2).

It is worth noting that in the above interpretation, the stages in the life cycle of my organism correspond very closely to those in the life cycle of *Theileria parva,* as worked out by Gonder.

From the above data the life cycle of the organism of Tsutsugamushi disease may be constructed as follows:

The rod, spheroid or ring-shaped bodies found intracellularly in lymphoid and red blood cells, represent an agamic generation. In progamic and gametic generations the organism is free in the blood plasma, assuming rod or ring shapes (Figs. 2-9), but forms shown in figures 4′, 5′, 6′, 7′, 10 are agamic. In the gametic generation it is transformed into an ameboid body (Figs. 11-17). During the metagamic generation the ameboid body comes to assume an oocyst-like appearance on account of the development of numerous small granules within it (Figs. 16-18). The oocystoid body then breaks down and sets free the granular inclusions, namely the metagametes. The metagametes (sporozoits) are the smallest units of the organism.

Theileria parva is found in the intestine or in the salivary gland of the tick during the metagamic generation. In my organism, however, this generation is also seen in the blood of the patient. In Tsutsugamushi disease, therefore, the mite may not be the necessary intermediate host. In this connection the possibility of infection with this disease by injection of infected blood is of much interest.

It is known that pirosomes multiply by fission. Arguing from analogy then, it is probable that the organism of Tsutsugamushi disease may also, under certain conditions, reproduce in a similar manner. As a matter of fact this organism often assumes a dumb-bell shape suggesting transverse division. If this crosswise division is really a process of reproduction, it should be counted as one of the peculiarities of this organism. The possibility undoubtedly exists that the larger type of dumb-bell shaped bodies appearing in the gametic generation may represent copulation of male and female elements (Pl. XI, Figs. 14, 15, 16, 17), instead of division. It is not possible to come to a definite conclusion on this point at present. The organism of Tsutsugamushi disease differs from Gonder's *T. parva* by its minute comma-shaped true sporozoit.

SUMMARY

1. Minute bodies, described as "rod," "spheroid" and "ring-shaped," have been found in the lymphocytes of lymph nodes and in mononuclear endothelial phagocytes of the spleen and lymph nodes, and in the region of the bite in patients suffering from Tsutsugamushi disease. They also occur free in the blood plasma, and in severe cases in the red cells.

2. The disease has been transmitted experimentally to monkeys, guinea-pigs, rabbits and calves. Bodies similar in appearance and distribution to those found in human cases, have been demonstrated in experimentally infected animals.

3. These bodies, on account of the difficulty of differentiating their cytoplasmic and chromatic elements, resemble bacteria, but no evidence of their bacterial nature has been obtained from cultural or animal experiments.

4. Cultural experiments have proved wholly negative.

5. Microscopically, these bodies are found to possess definite morphology, with parts comparable to the nucleus and cytoplasm of a cell. Moreover, the three principal types in which they appear (rod, spheroid and ring shapes), merge into each other. From these facts, it is concluded that the organism in question is a protozoon. Bodies of different sizes represent different stages in its life cycle.

6. Biologically, this protozoon is to be considered as a new species, resembling but showing differences from *Bartonella bacilliformis* and *Theileria parva*. The parasite to which it appears most closely allied is *Theileria parva*.

7. The organism found in Tsutsugamushi disease, I have designated tentatively as *Theileria tsutsugamushi*, spec. nov.

Here, I wish to express my sincere thanks to Professor A. Fujinami for his continued encouragement and help, and to Mr. Kuhara of Osaka for his generous aid in support of our work. A great deal of credit is also due to the assistants in my laboratory who have participated in the investigations.

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Explanation of Figures

PLATE IX

Drawn from smear preparations with camera lucida and Zeiss 1/12 apo. obj. and comp. oc. 6. Stained by the Giemsa method (Prowazek's trachoma granule stain).

A. Ring-shaped body. *B*. Spheroid body. *C*. Rod-shaped body. *a*. Small ring, and coupled rod and spheroid bodies. *a'*. Small ring-shaped body showing chromatic spot (resembling malaria ring form). *b*. Small spheroid body. *c*. Small rod shaped body.

Fig. 1.—Minute bodies embedded in large mononuclear endothelial cell.

Fig. 2.—Same in small mononuclear lymphoid cell, showing coupled rod and comma-shaped bodies.

Figs. 3, 4, 5.—Similar to Figure 2.

(The plates which are included here through the courtesy of the author are reprinted from the Japanese originals. There they bore the numbers I, II, and III which to fit the series in The Journal have been changed to IX, X, and XI.—Editor.)

PLATE X

Drawn from a smear preparation taken from patient's blood (First published in 1906, Hokuetsu Igakkai Zasshi, No. 156). Drawn from smear made from patient's blood; magnification and staining as in Plate IX.

Fig. 6.—*A*, large, *a*, small ring-shaped body. *B*, large, *b*, small spheroid body. *C*, large, *c*, small rod-shaped body.

Fig. 7.—Large and small rod-shaped bodies embedded in red blood cell.

Figs. 8, 9.—Large rod body, showing three chromatic spots.

Figs. 10, 11.—Small rod and ring-shaped bodies.

Figs. 12, 13.—Ring bodies resembling malaria-rings.

Fig. 14.—Large rod body.

Figs. 15-17.—Large gametocytes connected together by thin thread.

Figs. 18-31.—Smear from bite wound.

Fig. 18.—Rod bodies (*a*) showing achromatic area around chromatin substance. Also another small body with different morphology.

Figs. 19-21.—Rod bodies showing indistinct chromatin substance.

Figs. 22, 24, 26.—Same as figure 18a.

Fig. 24b.—Rod body with two chromatic spots.

Fig. 23.—Same with three achromatic spots.

Fig. 25.—Body with very striking achromatic area.

Figs. 27, 28, 31.—Two coupled gametocytes.

Figs. 29, 30.—Gametocytes; large spheroid body, same as fig. 6 B.

PLATE XI

Diagram of supposed life cycle of *Theileria tsutsugamushi.*

Figs. 1-22.—Free intercellular stage.

Fig. 1.—Sporozoites.

Fig. 2.—Rod-shaped bodies.

Fig. 3.—Elongated rod bodies.

Fig. 4.—Minute spheroid bodies.

Fig. 5.—Same as figure 3, but with an accessory small chromatic spot.

Fig. 6.—Enlarged spheroid bodies (macrogametocytes).

Figs. 7, 8, 9.—Same, like malaria ring body.

Figs. 4, 5, 6, 7, 10.—Spheroid bodies, resembling diplococci.

Figs. 11, 12.—Ring bodies assuming amoeboid appearance.

Fig. 13.—Same, enlarged form.

Fig. 14.—Large bodies coupled together.

Figs. 15, 16.—Large rod bodies, resembling trypanosomes. Note the two nuclei of different sizes and finely granulated protoplasm.

Fig. 17.—Large spheroid bodies, showing fine granulation (oöcyst formation).

Fig. 18.—Same, showing chromatic spot with achromatic ring.

Fig. 19.—Minute granulated body (Koch's free Plasmakugel).

Figs. 20, 21, 22.—Same, containing large granules.

Figs. 1a-16a.—Intracellular stages passed in lymphoid cells.

Fig. 1a.—Spheroid body embedded in mononuclear cell of lymph gland.

Fig. 2a.—Group of minute comma or rod-shaped bodies in mononuclear cell of blood.

Fig. 3a.—Same, showing grouping in mononuclear cell of lymph gland.

Figs. 4a-5a.—Same, showing less localized condition of the bodies.

Figs. 6a-7a.—Same, showing no localization.

Fig. 8a.—Rod and minute ring bodies in splenic cell.

Fig. 9a.—Same, in small round cell of lymph gland.

Fig. 10a.—Same as Plate IX, figure 1.

Fig. 11a-12a.—Rod, spheroid and ring bodies in large mononuclear cell of lymph gland. Fig. 12a also shows cell containing remnants of two rod cells.

Fig. 13a.—Similar to 11a.

Fig. 14a-15a.—Free large ring-shaped bodies identical with intracellular forms.

Fig. 16a.—Free ring-shaped bodies breaking down into minute granular bodies.

Fig. 1b-5b.—Intracellular stage in red blood cells.

Fig. 1b.—Group of minute comma-shaped bodies in red blood cell.

Fig. 2b.—Same, comma- or rod-shaped bodies slightly enlarged.

Fig. 3b.—Same, showing further enlargement of comma- or rod-shaped bodies and a few large rod-shaped bodies.

Fig. 4b.—Two large rod-shaped bodies adhering to surface of red cell.

Fig. 5b.—Ring-shaped body, and body showing formation of minute granulation.

Plate I

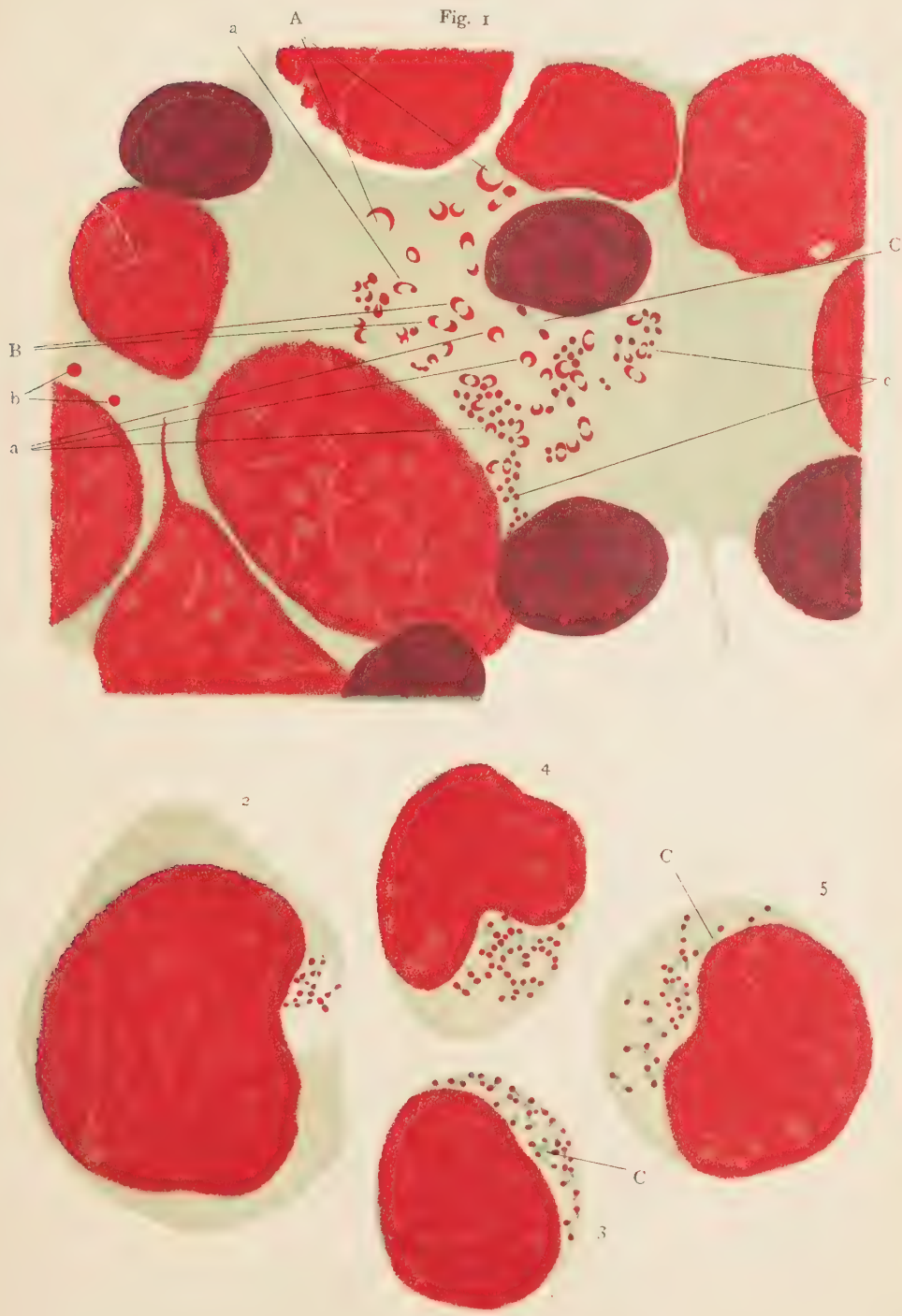


PLATE IX

Plate II

Fig. 6

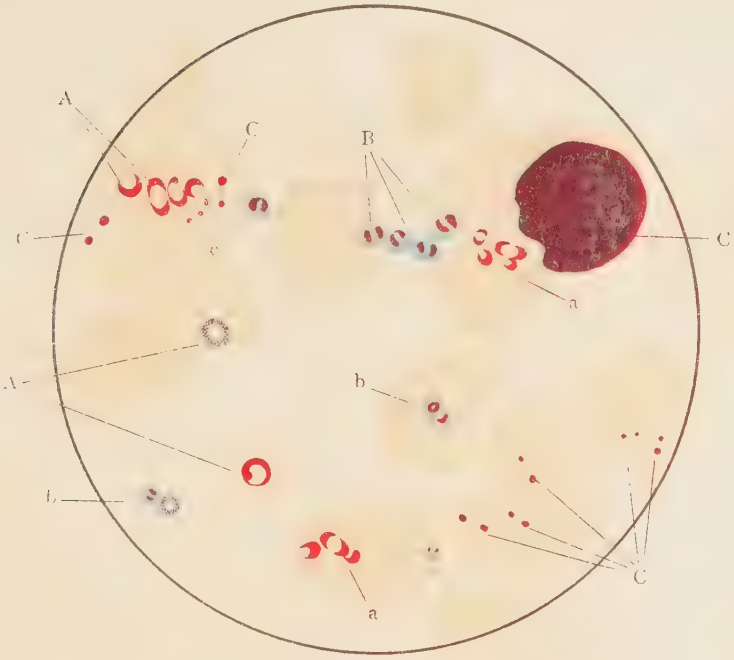


Fig. 7

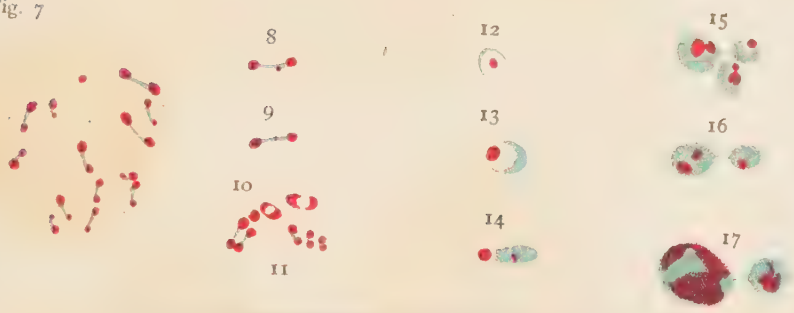


Fig. 18

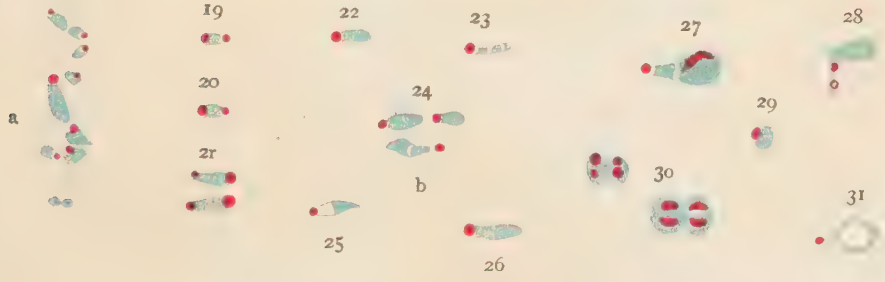
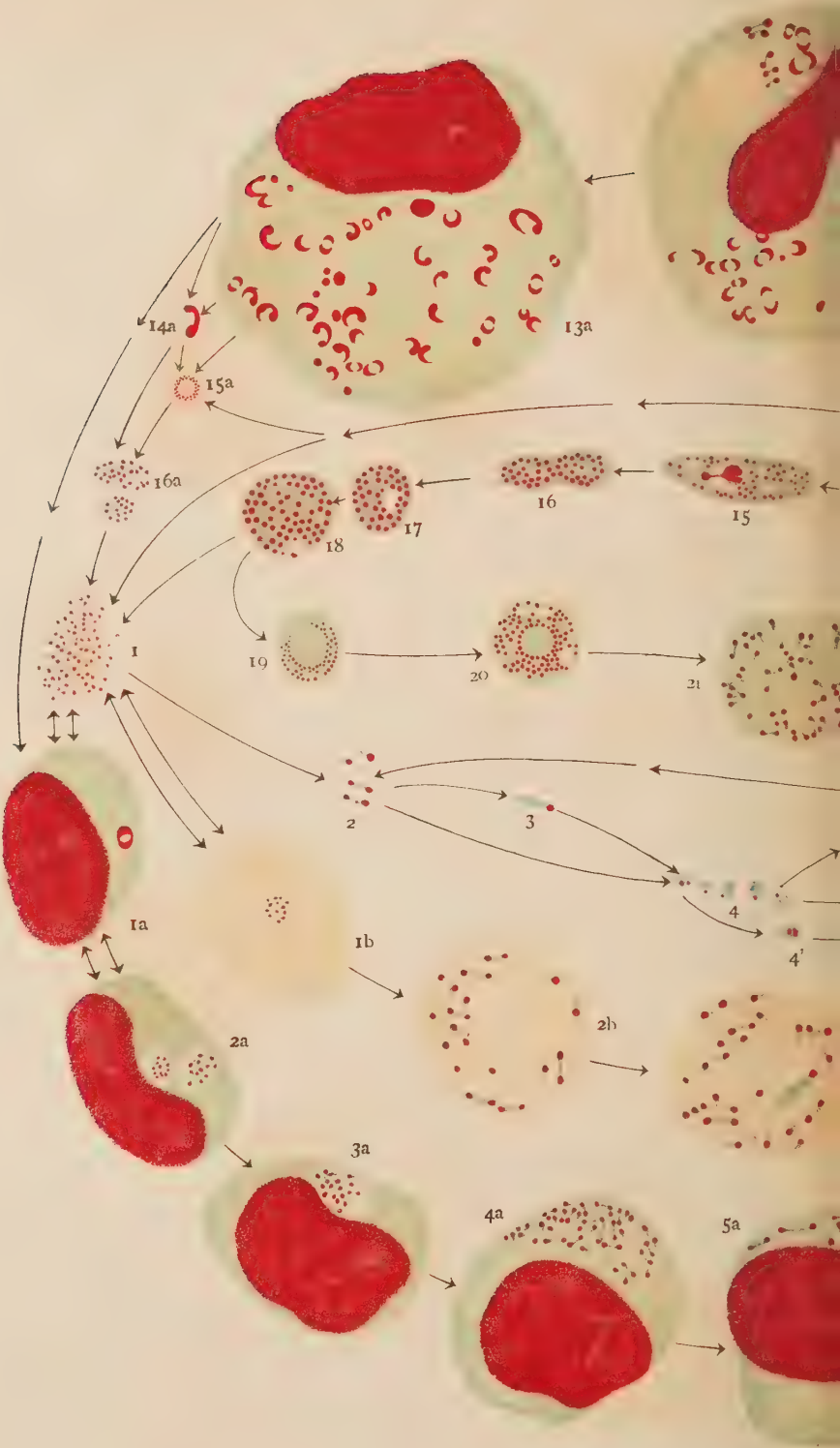


PLATE X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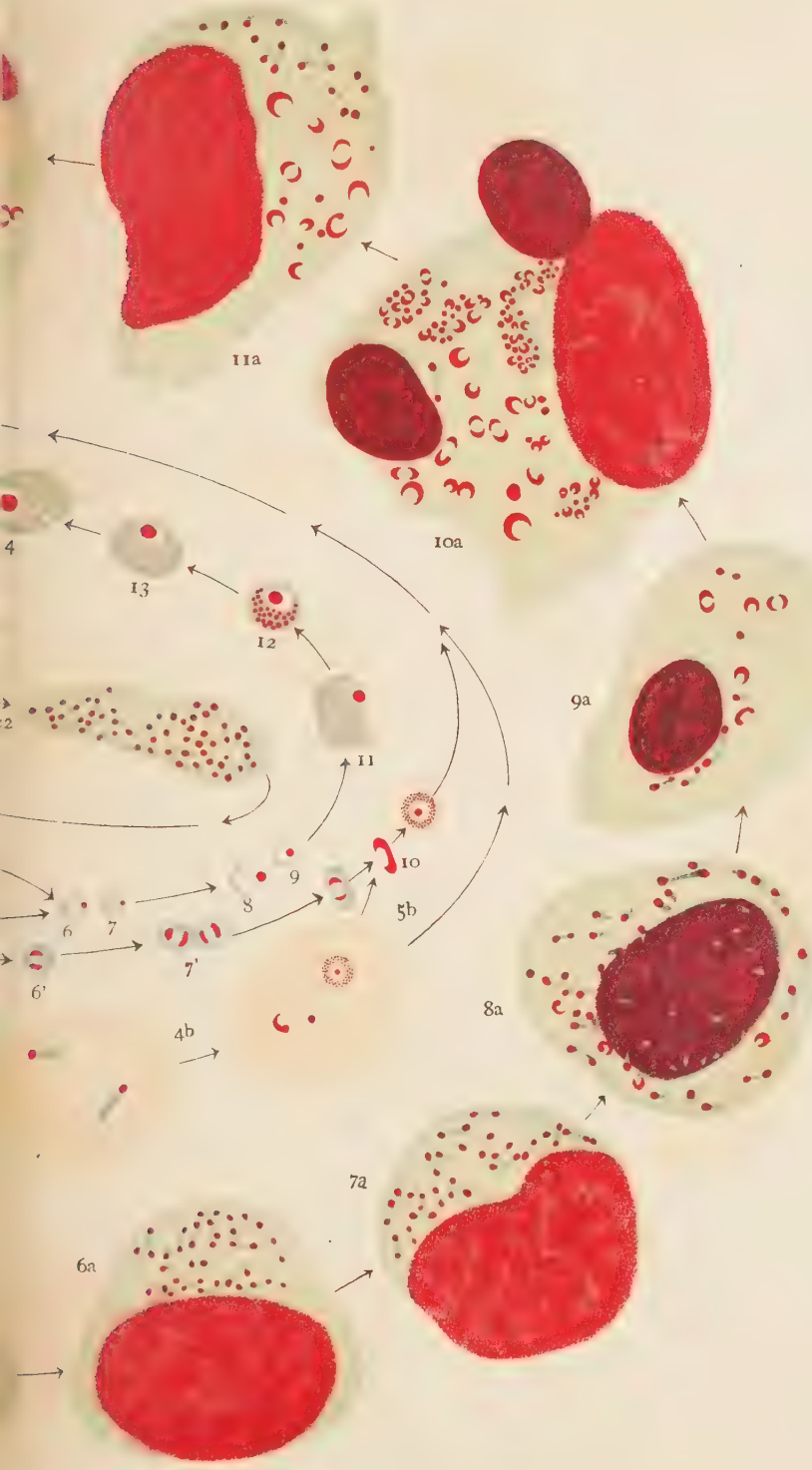
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6a

THE EGG LAYING HABITS OF CALIFORNIAN ANOPHELINES *

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During the months of May, June and July, 1920, the writers were stationed at Vina, Tehama County, California, in the central part of the Sacramento Valley, where a temporary summer laboratory was established for the purpose of investigating a number of problems concerning malaria and anophelines, particularly their egg laying habits, with which this paper deals. The information herein published will in a measure supplement, confirm and correct previous observations in this field.

The species dealt with were *Anopheles occidentalis* D. & K., *Anopheles punctipennis* Say and *Anopheles pseudopunctipennis* Theobald. The synonomy of the first mentioned mosquito, *A. occidentalis* D. & K., is somewhat obscure in that it was separated from *A. quadrimaculatus,* which it closely resembles, by Dyar and Knab in 1906, and its range was listed by them as "western United States, from Southern California to Alaska, eastward thru Canada to Maine." However, Howard, Dyar and Knab (1917) in their monograph point out as an additional note that Say's type specimen for *A. quadrimaculatus* has as its locality, "Northwest Territory," which discovery makes the four-spotted anopheline of the Pacific coast, *Anopheles quadrimaculatus,* submerging their name, *Anopheles occidentalis,* and making the Eastern species *A. guttulatus* Harris. This revised synonomy has not come into general use, however, and with the above explanation offered to avoid future confusion we shall use the name *quadrimaculatus* to refer to the Pacific coast species.

The mosquitoes used in the observations herein described were captured in shell vials at a number of stations in the vicinity, such as bridges, stables, outhouses and dwellings that were visited regularly each day. The captured mosquitoes were transferred to wide mouthed pint jars partly filled with water and covered with bobbinet. The jars were placed in rows on a glass shelf supported at its corners about six inches above the surface of a laboratory table. Thus elevated and resting upon the glass shelf an incandescent electric lamp could be placed directly beneath the jars for purposes of illumination and greatly facilitated observations from above, particularly in counting the eggs.

* Contribution from the laboratories of Entomology and Parasitology, College of Agriculture, University of California, Berkeley, California.

The water used in the jars at all times was taken from a deep well and showed a uniform alkalinity of 1840 parts per million expressed in parts of $CaCO_3$. The fauna and flora of the water was not determined but was practically negligible owing to the great depth of the bored well. Contamination that ensued later was undoubtedly uniform as all jars were concentrated in a small area not over 12 by 28 inches.

The temperature of the room in which these observations were made was taken on a recording thermometer. The record of this instrument showed at the expiration of the work that the maximum and minimum temperatures were 88° F. and 66° F., respectively, and that the average of each was 76° F. and 68° F. Here again there could have been no variation in the temperatures of the several jars.

Time of Deposition.—A total of sixty-five layings was recorded from May 17 to July 11. On thirty occasions we were able to obtain the exact or approximate time of oviposition. Of these, thirteen layings were made between nine and eleven in the evening, nine between eleven and daybreak, seven between sunset and nine p. m. and four during the afternoon. It must be understood, however, that artificial conditions may have seriously influenced these findings when it is considered that the light was never as intense in the laboratory as it would have been out-of-doors, that wind exercised little influence, and that the temperature curve showed a lag of approximately two hours as compared with out-of-doors as well as a distinct moderation.

The factor or factors governing the time of egg deposition are not known, but it would seem that light, temperature, humidity, and wind are probably important considerations. It is interesting in this connection that *Anopheles punctipennis* repeatedly deposited eggs in the full glow of a 40 watt tungsten lamp at a distance of about seven inches from the egg laying insect. It is safe to assume, however, that in these instances the insects oviposited in spite of the light conditions rather than because of them. That diffuse light or darkness is the normal condition during which eggs are deposited, is well illustrated by the fact that on June 3 the lights were left on from dusk until ten p. m., during which time there had been no oviposition. At that time the lights were turned off for ten minutes and at 10: 10 when the lights were switched on it was noted that three Anophelines had deposited eggs, none of them resuming until the light was again extinguished. The daylight records of oviposition appearing in our notes are without exception for overcast, humid days when the light intensity in the house approximated that normally occurring at dusk. The range of temperature between different oviposition periods was so great as to suggest but slight effect at best unless the stimulus should arise from

a change of temperature as from warmer to cooler and vice versa. Unfortunately, no humidity records were kept. Further work with this factor in consideration seems to offer a promising field.

Method of Oviposition.—Owing to the reluctance of *A. quadrimaculatus* to oviposit in the presence of light and the scarcity of *A. pseudopunctipennis* our observations regarding the actual process of deposition are limited to but one species, *A. punctipennis.* The only reference in the literature concerning this process that we have been able to locate is that of Kerschbaumer cited by Nuttall and Shipley (1902). Nuttall writes: "With the exception of Kerschbaumer (1901) nobody has claimed to have observed the process of oviposition. He witnessed the process but once in Anopheles, . . . he does not, however, describe the process (excepting in so far as he says the insect rested directly upon the water)."

On the evening of June 4 a specimen of *Anopheles punctipennis* was seen to behave in a rather excited manner, resting for a few moments on the surface of the water and then flying to the bobbinet or sides of the jar, remaining in each position only a few seconds. She finally came to rest for several minutes on the surface and assumed a position with the abdomen more or less parallel with the surface of the water, the wings held in the normal position with relation to each other but elevated at least the width of the body above the abdomen, the posterior end of which, comprising the last two segments, was tilted upward slightly. All six tarsi rested on the surface of the water, the middle pair being lifted above the body from time to time.

At 9:46 p. m. the first egg was deposited. This was accomplished by a rather nervous jerk of the abdomen following which an egg was seen to be protruding in a vertical position from the abdomen with its convex side directed to the rear. This position was held for four seconds when another convulsive downward twitch freed the egg from the abdomen, and as the latter was returned to its former position, another egg protruded and slipped instantaneously into the vertical position as the tip of the abdomen regained its original attitude. This procedure was continued for 19 minutes until a total of 174 eggs had been deposited. The deposition of the individual eggs took place at remarkably regular intervals of from six to seven seconds. During the entire operation the female remained motionless except for the monotonus jerking of the abdomen. At the conclusion of oviposition the mosquito remained without changing position for eight minutes, after which she slowly moved off to the side of the jar, scattering the eggs with her legs as she went. Numerous statements, based evidently on the remarkable patterns assumed by the eggs on the surface, appear in the literature regarding the method of placing the ova. Grassi,

quoted from Nuttall and Shipley (1901), stated that the eggs of *A. maculipennis* were deposited in pontoons, while those of *A. bifurcatus* were laid in star shaped patterns. In the above example, however, and in many subsequent observations of the same species, the eggs were seen merely to pile up in a heap beneath the insect, toppling over as the mass became top heavy and arranging themselves in various patterns dependent upon mutual adhesion and surface tension. At the time of deposition the eggs are pearly white, becoming progressively yellowish, then darker, until at the end of about thirty-five minutes the color becomes distinctly leaden, and in about forty-five minutes they appear dull black and under the microscope are a rich chitinous brown.

Number of Eggs Deposited.—Grassi, quoted from Nuttall and Shipley (1901), states that *A. maculipennis,* the European representative of our *quadrimaculatus-guttulatus* group, deposits 100 eggs, while Hindle (1914) dealing with the same species places the number at from 40 to 100. Howard (1900) referring merely to Anopheles (no species given) also gives the range from 40 to 100. Our observations point to a considerably larger number per laying. It is impossible at the present stage of our investigations to estimate the total number of eggs laid during the life of an Anopheline as we have not been able to start our series with bred females. Our experimental insects were invariably captured specimens concerning whose previous oviposition history we, of course, have no record.

Twenty-nine specimens of *A. quadrimaculatus* deposited thirty layings totalling 6,282 eggs, in lots ranging from 140 to 315 eggs each, bringing the average per laying to 209 eggs. Thirty-three females of *A. punctipennis* in thirty-three layings, ranging from 83 to 321 eggs each, deposited 6,700 eggs; making the average per laying 203 for this species.

Our records of oviposition in *Anopheles pseudopunctipennis* are extremely limited. We were able to obtain only four females during the course of the work. Of these, two oviposited, one a total of 157 eggs and the other but 55, bringing the average to 106 eggs per female.

Considering the females of all species under observation, 38.4% oviposited in captivity. *Anopheles occidentalis* females showed an oviposition percentage of 48.3%, *A. punctipennis* 31.2% and *A. pseudopunctipennis,* based on only four specimens, 50%. These figures are not, of course, indicative of what the particular species may do in natural surroundings as our specimens, as already stated, were captured females, unfed in captivity in the majority of cases and whose opportunities for feeding before capture were unknown.

It is pertinent at this point to make a statement regarding the number of batches of eggs deposited. In the course of the work we had several cases where the females oviposited on two consecutive nights. In such cases the two layings were recorded as one. One specimen, *A. quadrimaculatus,* no 61, deposited two true batches of eggs. In this case the female was captured under a bridge on June 9 and during the afternoon of June 12 she deposited 218 eggs. On June 13 she was given a meal of human blood and on June 19 deposited 140 eggs, dying on June 20. Both batches of eggs were hatched on the morning of June 15 and 21, respectively. On numerous occasions, dissections of females that had oviposited and been fed showed the ovaries completely filled with well developed eggs. Numerous observers have stated that Anopheles may deposit several batches of eggs with a single fertilization and a blood meal for each complement of eggs. The exact number of batches and the length of time over which they are deposited needs further observation. Accurate information on this particular point is highly desirable and might change present emphasis in control work.

The hazards of life in captivity probably affected oviposition, many dying thru accident by getting "spraddled" in the water before they were ready to oviposit. Fully fifty per cent. of those dying without ovipositing showed the presence of complements of well developed eggs upon dissection. The average length of life for unfed *A. quadrimaculatus* in captivity, disregarding their probable length of life before capture, was 4.5 days and for the fed specimens 8.5 days. For *A. punctipennis,* the length of life for unfed specimens under the same conditions was 4 days and with food, 6.3 days.

Morphology of the Eggs.—In comparison with the extensive work that has been done on the morphology and classification of the other stages of the anopheline life cycle, little has been done with the eggs. The ease of classification (at least for the three Californian species) by means of egg characters recommends this line of study to workers in other localities. The characters found to vary in such a manner as to make identification simple, are length of the egg, and position and length of the float. The consideration of one or more of these factors is sufficient to place the egg of local species correctly, but in a larger group it would be necessary to utilize other characters, such as the "frill" of Stephens and Christophers (1908), a feature omitted in most of the illustrations of anopheline eggs, which encircles the flat or upper side of the egg, the formation of the floats, or the reticular membrane enclosing the egg.

These authors classify anopheline eggs into three groups: 1. those with the lateral floats not touching the margin; 2. those whose lateral

floats overlap the upper side of the egg; 3. those without floats. According to this classification *A. quadrimaculatus* and *A. punctipennis* fall in the second class and *A. pseudopunctipennis* in the third.

The egg of *A. quadrimaculatus* is fusiform, slightly rounded at each end, and tapering to the extent that one end is slightly broader than the other. The upper surface is flattened with a slight longitudinal concavity while the lower surface is broadly convex, the convexity becoming more pronounced at the broad end of the egg. The upper surface is granular, bordered by a laterally striated frill 16μ in width, except at the floats, while the lower surface shows, under proper light, a silvery reticulation. Medianly placed are two roughly oval lateral floats, each divided in a majority of cases into twelve scalloped compartments. The larger part of the area covered by these floats is on the lateral faces of the egg, but they project dorsally over the margins which are described as "gunwales" rather aptly by one author who

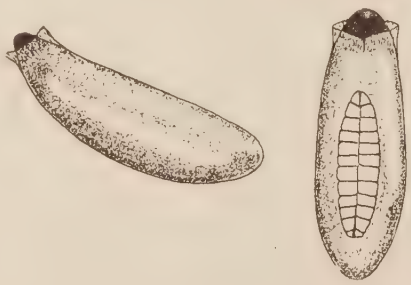

Fig. 1.—Illustrating the egg of *Anopheles pseudopunctipennis* Theobald. At the left, lateral view, and at the right, dorsal view.

likens the egg to a boat. The eggs range in length from 592 to 656μ. The floats vary in length from 144 to 224μ.

The eggs of *A. punctipennis* resemble those described above with these exceptions, the upper flattened surface is distinctly concave longitudinally, the floats are decidedly wider, projecting dorsally over the margins to the extent that they more nearly meet on the dorsal median line than those of *A. quadrimaculatus*. The floats also include approximately eighteen scalloped compartments each and extend along the sides for slightly more than one-half the entire length of the egg, while in *A. quadrimaculatus* the floats extend for only one-third the length. The range in length of the eggs of *A. punctipennis* extends from 544 to 576μ while the float length remains fairly constant at 320μ.

In *Anopheles pseudopunctipennis* is found a peculiar specialization represented in the general characters of the egg of *A. turkhudi* Liston, which is placed by Stephens and Christophers (1908) in class three of their table as lacking floats, altho vestiges are present. The eggs

are shorter than either of the two already mentioned, ranging from 512 to 528μ. The upper surface is nearly flat, showing little concavity longitudinally altho the lower surface shows a marked convexity. Both ends of the egg are rounded, one being considerably broader than the other. The floats are represented by a fusiform closely appressed area, approximately 270μ long, lying on the dorsal side of the egg and nearer the blunt end. This area is divided medianly by a line which is assumed by the authors to be the line of contact of the two floats that have been forced up from the sides. Lateral lines mark off each longitudinal half of the area into twelve sections representing the twelve original compartments of lateral floats. This area is so appressed that its position is not distinguishable from a lateral view.

Near the narrow end of the egg the membranous covering flares out from the body of the egg to form a translucent, striated collar which completely encircles the end, with the exception of a triangular incision down the dorsal median line in a manner which reminds one of an "oversized dress collar" (Fig. 1). The egg hangs at an angle in the water, supported by surface tension on this "collar." The larvae, however, unlike those of *Anopheles turkhudi,* whose eggs those of *pseudopunctipennis* resemble, retain the horizontal position at the surface of the water.

Selection of Breeding Places.—Much has been written regarding the type of breeding places frequented by various species of Anophelines and experienced observers in this field are able to forecast with a high degree of accuracy the species that they will find breeding in a given situation. This intuition is almost impossible to analyze and attempts to work out the ecology have yielded as yet only partial explanations. Disregarding the causes that make particular pools acceptable or unfavorable for the life of the larvae, there remains a fundamental question on which the whole study depends. This is, the determination of whether the selection of the particular pools is due to selective oviposition on the part of the female or the inhibiting effects, chemical or biological, upon the larvae present in some pools which are unfavorable to the species under consideration.

In the course of our work we found pools from which there constantly emerged, in the one case *A. quadrimaculatus* and in the other *A. punctipennis* with no mixture of the species. These pools were therefore classified as *quadrimaculatus* pools or *punctipennis* pools. Eggs of each species were "planted" in the pools hitherto inhabited only by the larvae of the other species and their development observed. In order to accomplish this under the most natural conditions, "lug" boxes with bobbinet coverings substituted for bottoms were inverted in the pools, supported a little from the bottom by stakes

and reaching an equal distance above the surface to prevent overflow. By supporting them a little from the bottom it was hoped that the natural enemies might successfully enter and that the enclosed water would partake of all the conditions prevalent in the pool and still not allow the escape in any appreciable numbers of the surface feeding larvae. Through an opening in the bobbinet covering, the eggs were gently washed on the surface of the water in the box. Unfortunately our boxes were of necessity located in pools subject to the rise of a creek, an occurrence that happened several times in the night owing to thunder storms in the mountains and the unexpected flow of unused irrigation water. Due to this contingency all of our boxes with the

Fig. 2.—Showing manner in which lug boxes were placed in a typical *A. punctipennis* pool to determine suitability of this pool to other species of anophelines.

exception of one set were found on one or more mornings to be awash, rendering their results problematical. One set, however, was conducted under optimum conditions. A pool, six by twenty-five feet in area known in our experiments as a *punctipennis pool,* was formed by the receding creek mentioned above and fed by seepage and a trickling connection with the main stream. It was shaded, cool and thickly overhung with surrounding brush, mainly grape, cottonwood and sycamore. The bottom was made up of water-rounded stones ranging in size from pebbles to small boulders and its prominent vegetable growth was a member of the Crenothrix group. The water was unusually clear and had an alkalinity of 840 parts of $CaCO_3$ per

million. It showed evidence of being permanent, in part at least, throughout the summer. Two boxes were installed as mentioned above (Fig. 2) enclosing a section in which larvae had been observed and removed. On June 28, 474 eggs of *A. quadrimaculatus* were placed in one box and on July 1, 635 eggs of *A. punctipennis* were placed in the other. These eggs were observed to hatch in the normal period in both boxes and daily observations proved their gradual development, pupation beginning on the thirteenth day after egg deposition. No accurate count of the numbers emerging was possible under the existing conditions, but from general observation, no retardation in development or diminution in the expected numbers of *A. quadrimaculatus,* altho breeding in an *A. punctipennis* pool, could be noted. The results of this experiment left to our minds only two alternatives in the question of selective breeding places—either the *punctipennis* larvae are cannibalistic on the *quadrimaculatus* larvae when the former are in optimum surroundings, and the process is reversed when optimum conditions are furnished to *quadrimaculatus* larvae, or what is far more likely—the female exercises selection in depositing her eggs. Several experiments were inaugurated to settle this first alternative by mixing the eggs in one box but the floods mentioned above rendered our results untrustworthy.

Incubation Period.—In the regular routine of laboratory work, the jars were examined every morning at about nine o'clock, the eggs that were found for the first time being entered as deposited for that day and those found to be hatched were entered at the same time. By observing this routine the average incubation period was very nearly approximated. For *A. quadrimaculatus* the average incubation period was 2.5 days with a range from 2 to 4 days. The eggs of *A. punctipennis* averaged 3.2 days with a range from 2 to 6 days. Temperature is quoted by many authors as distinctly influencing the length of the incubation period. With many of our sets, however, laid on the same day and subjected to the same conditions a considerable amount of variation was recorded. It seems highly probable that temperature should exercise a decided effect on incubation particularly at the extremes but within a certain range such as our eggs were subjected to, i. e., 68° to 76° F., little effect could be noted.

Desiccation Experiments.—Mitchell (1907) states that Dr. Dupree "has had the eggs of *Anopheles* develop after as many as ten hours out of water, this, however, being exceptional." Stephens and Christophers (1908) state anopheline eggs removed from water, placed on paper and allowed to dry for more than two, or, at the most three days, will not hatch if they are kept at a temperature of 86° to 96° F. In an attempt to check these findings for the Californian species, eggs

of *A. quadrimaculatus* and *A. punctipennis* were removed from the water six hours after they had been deposited by allowing them to adhere to the surface of a strip of filter paper dipped among them, leaving a number of eggs in the jar as a check. The filter paper was then suspended by pins inside a capsule box and allowed to dry out at room temperature. Drying was accomplished in a remarkably short time for at the end of four hours the paper was entirely dry and the eggs rattled off its surface at the least movement. At intervals of twenty-four hours a supply of dried eggs were taken from the filter paper and placed in shell vials of tap water. We were never able to obtain a hatch from eggs of *A. punctipennis* that had been removed from water for twenty-four hours. However, with *A. quadrimaculatus* eggs removed from the water on June 14, dried and replaced on the fifteenth, sixteenth and seventeenth, having been subjected to drying for 24, 48 and 72 hours, respectively, there were produced excellent hatches on the seventeenth, eighteenth and nineteenth, showing not only that the eggs of this species can withstand drying for these periods, but also the rather interesting fact that egg development ceases as soon as they are removed from the water. Eggs from this lot placed in water on the eighteenth (96 hours of drying) and for several succeeding days failed to hatch. The maximum and minimum temperatures to which the eggs were subjected during this period were respectively 74° F. and 65° F. Another attempt to duplicate this set of experiments with the same species and technique when the temperature ranged between 70° F. and 80° F. resulted in the failure of the eggs to hatch after 48 hours of drying.

The authors present this paper not as a complete treatise on egg deposition of Anophelines but as observations that may add to the general fund of information concerning these insects whose activities are of such vital interest and importance to mankind in all parts of the world. The authors wish to acknowledge the helpful co-operation and limitless enthusiasm in this work on the part of their two student assistants, Mr. Clifford T. Dodds and Mr. John F. Lamiman of the University of California.

SUMMARY

1. The process of egg deposition in *Anopheles punctipennis* is described.

2. The number of eggs deposited per laying is found to be greater than hitherto recorded, *A. quadrimaculatus* averaging 209 eggs and *A. punctipennis* 203 per laying.

3. Descriptions are given of the eggs of the Californian anophelines whereby they may be differentiated, including a description of the

egg of *A. pseudopunctipennis,* which represents a marked departure from the usual anopheline type.

4. Observations are introduced to indicate that specific breeding places are due to selective oviposition.

5. The incubation period of the eggs of *A. quadrimaculatus* is 2.5 days, and *A. punctipennis* 3.2 days, and *A. pseudopunctipennis,* 3 days.

6. It was found that the eggs of *A. quadrimaculatus* could withstand drying for 72 hours but that those of *A. punctipennis* failed to hatch after 24 hours of drying.

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ON THE MIGRATORY COURSE OF *TRICHOSOMOIDES CRASSICAUDA* (BELLINGHAM) IN THE BODY OF THE FINAL HOST*

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Trichosomoides crassicauda, a nematode belonging to the family Trichinellidae, was found in the bladder of wild rats in 1845 by Bellingham. Hall (1916) has summed up the previous work on this species. This nematode is remarkable on account of the great difference in size between the males and females. The male is commonly found parasitic in the vagina or uterus of the female, having a length of 1.46 to 2.5 mm. and a width of 23 to 33μ. The female is 10.5 to 13 mm. long and attains a maximum width in the posterior region of the body of about 0.2 mm. Nothing is known of the method of infection of this parasite or its migratory course to the bladder of the rat. Von Linstow (1874) suggested that the embryos might bore into the wall of the digestive tract and make their way to the pelvis of the kidney by way of the renal artery. He described also that sexually immature individuals, the males but little smaller than the females, were found in the pelvis of the kidney and that copulation took place in the ureters. Later the females became larger and the males entered their vaginae. Hall (1916: 16) found that the embryos escaped from their shells in the vagina of the female after the worm had been in normal salt solution a short time. He suggested from this observation and from the fact that such embryos seemed to live only a short time that infection must take place in a rather short period as a rule, or else the embryos would perish. He also described that the embryos which had just escaped from the egg, had a body of almost uniform thickness, terminating in bluntly rounded ends, while von Linstow stated that they were provided anteriorly with a single lancet-like process.

I found some specimens of *Trichosomoides crassicauda* in wild rats *Epimys norvegicus,* collected near Baltimore during the winter of 1919-20. The parasites were usually found attached to the wall of the bladder with their anterior ends somewhat embedded in the mucous membrane. They cause a rather high degree of catarrhal cystitis according to the number of the parasites present. The mucous membrane of the bladder was found congested and swollen and the urine

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more or less muddy. The number of the parasites in the bladder of a single rat is commonly small, although sometimes ten or more specimens were found in a single host.

In order to discover the method by which the rat is infested with *Trichosomoides crassicauda* I fed two white rats on January 20, 1920, with the eggs which contained the fully developed embryos. One of these rats became infected and the parasites developed to maturity. The eggs from the urine of this rat were used in later experiments. After the success of this experiment I undertook to investigate the migratory course of the larvae in reaching the bladder of the rat by feeding white rats with large numbers of the eggs of this parasite, and examining the various organs a short time after feeding. Three rats were used at different times but from only one of them were the larvae recovered. The results of this study are incomplete and a much larger series of experiments will be needed to clear up the details of the problem. However, some very definite information was obtained in regard to the migratory course of this parasite in the body of the final host. Since it will be impossible for me to continue these experiments I am writing up my results in hopes that some one else may use them in carrying on further work on this interesting problem.

Since the methods used with the three experimental rats were the same, I will describe here only the details of the work with the one rat which gave positive results.

On May 15 and 16, I fed one half grown white rat with many eggs of *Trichosomoides crassicauda* collected from the urine of the white rat experimentally infected with the parasite and of two wild rats which were infested naturally. The next day I fed it 15 adult worms containing many eggs in their uteri and also many eggs collected from the urine of the three rats mentioned above. On the following day also it was fed with many eggs collected from the urine of these rats. I killed this rat on May 19, one to four days after the various feedings.

The following technic was used in the recovery of the larvae from the body of the rat. First I opened carefully the abdominal cavity, avoiding bleeding, and washed it out several times with normal saline for the purpose of collecting any larvae contained in it. The liver and other internal organs were found to be a little congested without showing any bleeding points. Then I opened the pleural cavity in the same way and washed it out with normal saline. Both lungs were found to be a little congested showing some bleeding points on their surfaces. I examined one third of the liver, both kidneys, the spleen, and the left lung according to the following method. These internal organs were crushed into fine pieces and washed with normal saline

two to four times, and filtered through a fine wire net. The filtrate from the different organs and wash water of abdominal and pleural cavities were kept carefully separate and were centrifuged to collect the larvae.

The following results were obtained from these examinations. Four larvae were found in the abdominal cavity, two in the pleural cavity, and three in the left lung. One third of the liver, both kidneys and the spleen were examined without finding any larvae, while the right lung and two thirds of the liver were preserved for later microscopical examinations. I also examined the ureters, bladder and heart, without finding any larvae. Comparing the structure of the young larvae found in the body cavity and in the lungs with larvae just escaped from the eggs, I learned the following facts. The larvae just from the eggs have a very small body of almost uniform thickness, terminating in bluntly rounded ends. They measure about 0.21 to 0.25 mm. in length and 8 to 10μ in thickness. It was impossible to make out any details of internal structure at this stage. The larvae found in the abdominal cavity of the experimental rat measured 0.82 to 0.84 mm. in length and 34 to 35μ in width. The anterior part of the body was a little thicker than the posterior end and ended bluntly. The digestive tract was not very clearly defined. The posterior end of the body was blunt with a diameter of about 0.02 mm. and had a small depression. The esophagus consisted of about twenty irregularly shaped cells. No esophageal cells were present for about 0.02 to 0.025 mm. at the anterior end. The esophagus was relatively long, measuring about 0.28 mm. The intestine ran straight along the middle body toward the posterior end and consisted of cells containing fine granules. The larvae found in the pleural cavity and two of the larvae found in the lungs were larger than those from the abdominal cavity. They were about 2.34 mm. long and 0.1 mm. thick. The anterior end was rounded and 0.03 mm. in diameter. The width increased toward the middle of the body with a maximum of 0.1 to 0.11 mm. and then decreased posteriad. The esophagus consisted of about twenty cells and measured about 0.54 mm. in length. No cells were present for a short distance at the anterior end. One larva found in the lung was smaller than the others found in the same location, having a similar size and structure to the larvae found in the abdominal cavity. It is very probable that the larger larvae are females and the smaller ones males.

From these experiments I learned the following facts (1) Infection with *Trichosomoides crassicauda* can be induced in the white rat by feeding the eggs of the parasite. (2) The adult worms found in the bladder of experimental animals are very few compared with the

number of eggs swallowed. (3) The eggs swallowed by the final host hatch in the digestive tract and penetrate through its wall into the abdominal cavity. From here they travel into the pleural cavity probably through the diaphragm and penetrate into the lungs from their surfaces.

The passage of the larvae of *Trichosomoides crassicauda* into the lungs of the experimental rat is very interesting in relation to the recent work on the life history of *Ascaris lumbricoides* which proves that after hatching in the intestine of the final host the larval stages of this parasite must make their way to the lungs before they can complete their development. Yoshidi (1918) and Ransom and Foster (1920: 30) conclude that this phenomenon is of common occurrence in the life cycles of parasitic nematodes. Besides the hookworms, Strongyloides, *Ascaris lumbricoides, Ascaris suum* and *Belascaris marginata,* they note also that *Haemonchus contortus* from the sheep and *Ascaris anoura* from the python probably have a lung phase in their life cycles. Recently Neshi (1918) reports the finding of four larvae of *Trichuris depressiuscula* in the lungs of a dog twenty-one hours after experimental infection.

This observation and my finding the larvae of *Trichosomoides crassicauda* in the lungs of the experimental rat after feeding with the eggs add the family Trichinellidae to those in which this phenomenon occurs, and strengthens Ransom and Foster's hypothesis that parasitism of the lungs by nematodes is a more primitive condition than parasitism of the alimentary canal.

On the method of migration of the larvae of this nematode from the lungs to the bladder of the host there is little definite information. Von Linstow's (1874) finding young worms in the kidneys and ureters suggests that they make their way to the kidneys and then pass down the ureters to the bladder. How they make their way from the lungs to the kidney is still an unsolved question. Taking for granted that the migration to the lungs is a necessary phase of the life history of this parasite, there are three possible ways in which it might migrate from the lungs to the kidneys. (1) The larvae might make their way into the small branches of the pulmonary veins, be carried to the heart and then pass to the kidney along the aorta and renal arteries. (2) The larvae in the lungs might break into the air cells and, like the hookworm larvae, travel up the trachea and down the esophagus into the intestine. From here it would be necessary for them to make their way to the kidneys by way of the body cavity. (3) Finally the larvae in the lungs might make their way back into the pleural cavity, through the diaphragm and body cavity to the kidney. The course by the blood stream would seem to be very difficult if not

impossible on account of the large size of the larvae and the fact that the renal artery is a small vessel which branches at right angles from the dorsal aorta. It is possible of course that the smaller type found might follow this course. It seems to me that the second course is the most probable, but the solution of this interesting problem must await future investigations. At any rate the passage to the kidney requires a prolonged and difficult migration. This may account for the difficulty of producing the infestation and why the number of worms found in the bladders of the wild rats is usually so few.

SUMMARY

1. The infestation of rats with the bladder nematode, *Trichosomoides crassicauda,* was accomplished by feeding the eggs.

2. The finding of larvae of this species in the body cavity, pleural cavity and lungs of an experimental rat fed with large numbers of eggs, suggests that in the life cycle of this species the larvae must pass to the lungs before they can establish themselves in their normal habitat.

3. This observation and other recent studies strengthen the view that migration to the lungs is a common phenomenon in the life cycle of nematodes.

4. How larvae reach the bladder from the lungs was not determined but they probably are not carried in the blood vessels.

I wish to express here my thanks to Dr. W. W. Cort, under whose direction this work was carried on, for his help in the course of this investigation and for revising the manuscript.

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NOTES ON *NOSEMA APIS* ZANDER*

R. Kudo

Aside from *Nosema bombycis* Nägeli, no other Microsporidian has received so much attention of investigators as *Nosema apis* Zander. A disease of adult honey bees for which the Microsporidian is responsible, and which is known by different names such as Nosema-Seuche, Isle of Wight disease, Nosema disease, etc., has been reported to occur in various parts of the world. In North America, White (1914) found 40 infected bees in 120 samples received from 27 states of the union, and two diseased samples received from Canada. The same author further published (White, 1919) valuable results of experiments on the means by which the spores may be killed.

The morphology and development of the Microsporidian have, however, been studied by but three investigators, i. e., Zander (1911) and Fantham and Porter (1912). According to these authors, the spores of *Nosema apis* are on the whole similarly constructed to those of *Nosema bombycis* studied by Stempell (1909). Recently, I had an opportunity of studying the Microsporidian, and have obtained more or less different results by observations upon the structure of the spore from those of the above mentioned European investigators. I shall briefly describe the results in the following pages.

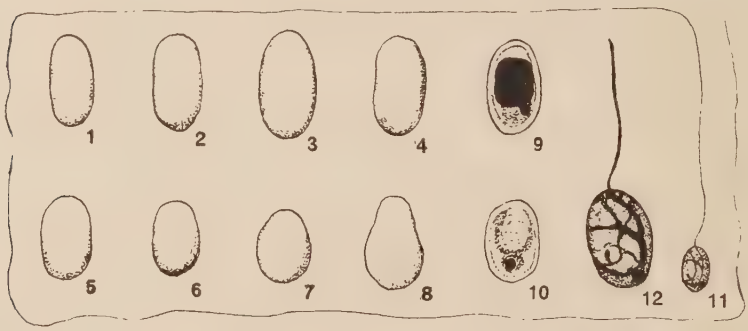
In a field at Spring Valley, New York, 660 workers of *Apis mellifica* were collected from August 27 to September 5, 1920. Although I could not trace the hive for these captured bees, there were a number of hives in the nearby woods where the collection was made. The digestive tract of each bee was drawn out from the body by means of a pair of forceps and a needle. A part of the ventriculus was smeared on a slide and examined microscopically in the fresh state.

Out of 660 bees thus examined, 25 were found to be infected by *Nosema apis*. After encountering one or two heavily infected bees, it was not difficult to diagnose a heavily infected host insect by its inactivity and peculiarly softened abdomen. When a part of a heavily infected ventriculus was placed on a slide, the milky white appearance gave a definite sign of the nature of the infection before a microscopical examination was made. Four bees contained spores similar to those of *Nosema apis*, differing, however, in larger dimensions and a large clear rounded space at one extremity. The presence of a

* Contribution from the Zoological Laboratory of the University of Illinois, No. 167.

space which is generally called a vacuole, in the mature spores, recognizable in fresh state, has commonly been noted in many species of Microsporidia. Frequently the spores of *Nosema apis* show also a clear rounded area near at one end, which, however, does not occur so regularly as in the spores from the above mentioned four bees. Whether this form should be distinguished from *Nosema apis* by a different specific name or not will have to be determined by a comparative study on the various stages of their development.

The dimensions of spores of *Nosema apis* differ somewhat widely according to different authors. According to Zander (1911), the spore meausres 5μ in length and 2.86μ in breadth, and the polar filament seemed to be shorter than that of *Nosema bombycis* measured by Stempell (1909). Fantham and Porter (1912) recorded the length and breadth 4 to 6μ (up to 7μ) and 2 to 4μ, respectively, and stated



Spores of *Nosema apis*. Figs. 1-8. Fresh spores of various form and size. × 2350. Fig. 9. A spore stained with Heidenhain's iron hematoxylin. × 2350. Fig. 10. A spore stained with Giemsa's solution. ×2350. Fig. 11. A spore with an extruded polar filament, stained after Fontana (the polar filament is diagrammatically shown without changing its approximate length). × 1200. Fig. 12. A more highly magnified view of the spore shown in fig. 11. ×2350.

that the polar filament was "about 60μ" long. White's measurements (1919) are as follows: fresh spores in India ink smears, 4.46μ long by 2.44μ broad; stained spores, 4.15μ long by 2.06μ broad.

According to my observations, fresh mature spores vary from 4.6 to 6.4μ in length, and from 2.5 to 3.4μ in breadth and thickness. The dimensions were obtained by measuring 250 spores. As mature spores (Figs. 1-8) differ to a more or less great degree in form and size, care has been taken in choosing the spores. I have measured ten spores from a smear of each infected individual, one spore taken at random near the center in every other field.

In the fresh condition, a spore does not show any differentiation in its contents. It is slightly less refractive than that of *Nosema bombycis*. The polar filament is invisible in fresh state, as in the

majority of microsporidian spores, except such a form as *Thelohania magna* (Kudo, 1920). The presence of a polar filament in the spore, therefore, could only be proved by causing its extrusion. Zander (1911) made no effort, and mentioned simply that it seemed to be shorter than that of *Nosema bombycis* as studied by Stempell. Fantham and Porter (1912) stated that it measured about 60μ in some spores they had examined.

The polar filament of *Nosema apis* can easily be extruded and observed, although some authors are inclined to think it is "very difficult of observation under any circumstances" (Fantham and Porter, 1912: 174). Either mechanical pressure (Kudo, 1913) or perhydrol (Kudo, 1918) causes extrusion of the filament in fresh spores. For quick observation a dark field microscope is indispensable (Kudo, 1918). But for permanent preparation, Fontana's staining (Kudo 1920) gives best results according to my more recent observations.

As to the method of application of mechanical pressure upon the spores, I stated briefly (Kudo, 1913) that they were pressed by fingers between a cover glass and a slide. Since I have often been asked as to the exact technique, I shall briefly describe the method here. A very small drop of water emulsion of fresh microsporidian spores is placed on a slide by means of a fine capillary pipette and is covered with a cover glass. It is desirable to have the outer margin of the cover glass unoccupied by the emulsion. Place the slide on a smooth and steady surface, and cover the cover glass with a piece of cloth or filter paper, over which the elbow is gently applied. Give a strong downward push to the arm. This will instantly cause the extrusion of polar filaments. The slide now can be brought on the stage of a dark field microscope and examined. To make the preparation permanent the cover glass must be lifted up carefully. After being fixed with fixative such as sublimate alcohol, the smear is stained after Fontana.

The completely extruded polar filament of spores of *Nosema apis* measures from 230 to 280μ in length. The remarkable difference in the length of polar filaments compared with that obtained by Fantham and Porter, is due solely, I believe, to the difference in the technique applied. In the case of *Nosema bombycis,* I have already shown (Kudo, 1913, 1916) how different methods bring out entirely different results regarding this delicate structure characteristic of Microsporidia. Iodine water has been used by several investigators, including the above mentioned English authors, in causing the filament extrusion of microsporidian spores. Yet evidences obtained by comparative studies of various methods have shown me that the iodine water in this case is not likely to cause the extrusion, but simply stains the already extruded

polar filaments, most probably due to the pressure of the cover glass, to a more or less recognizable extent, showing, therefore, only incompletely extruded polar filaments in very small numbers.

When examined under a dark field microscope (Fig. 13), the extruded filament of *Nosema apis* shows a uniform thickness throughout, as those of other Microsporidia which I have studied up to the present. Its form is, however, very striking in some cases. Leaving the spore at one end, it shows about from 10 to 15 undulating courses of a large wave length. This part is followed by another about 10 to 15 turns of uniformly small wave length. Somewhat similar conditions were frequently noticed in the polar filament of *Nosema bombycis*, extruded under the influence of perhydrol (Kudo, 1918, Fig. 5), but it was not so conspicuous as in the present Microsporidian. As far as I am aware, this peculiar condition has never been reported to occur. This wavy form becomes straightened out gradually and loses its former appearance, although the distal portion often remains in

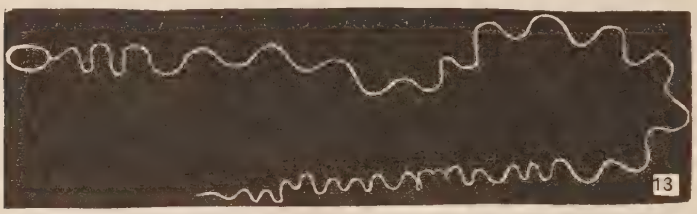


Fig. 13. A spore of *Nosema apis* with the extruded polar filament viewed under a dark field microscope. ×1200.

more wavy condition than the basal. Some polar filaments are, however, as straight as a stretched thread. The difference in the form of the extruded filament is due, in my opinion, to the difference in pressure received. It seems probable that when the spore receives a sudden violent pressure, the polar filament escapes from the spore without unwinding its coiled form, and that when the spore receives a gradually increasing pressure, the extrusion of the filament takes place slowly in a more or less straightened form. When the cover glass is removed from the slide, the agitation of the emulsion in which the spore is floating, apparently tends to straighten the filament, so that after Fontana's staining straight forms tangled in various ways are mostly to be found. Fontana's method stains not only the extruded polar filament, but also its unextruded portion. In this regard, the method has superiority over Löffler's (Kudo, 1913). Thus in the spore shown in Figs. 11 and 12, the unextruded part of the filament is clearly distinguishable, suggesting the extrusion in this particular spore was incomplete.

When the spores are fixed and stained, the contents appear more or less differentiated. In spores stained with Giemsa's solution, a small rounded sporoplasm taking a typical blue color with its deeply red nucleus, is seen near one end, while between this and the other end of the spore, the polar capsule with its polar filament is observable (Fig. 10). In spores stained with Heidenhain's iron hematoxylin, similar differentiation appears, although the polar capsule with its filament frequently stains as deeply as the nucleus of the sporoplasm (Fig. 9). The polar filament does not start to coil close to the end of the spore, to which it is attached. The basal portion is rather straight and begins to coil some distance back (Figs. 9 and 14). Zander (1911) observed this portion, but misinterpreted the relative position of the polar filament and the sporoplasm. Thus the structure of

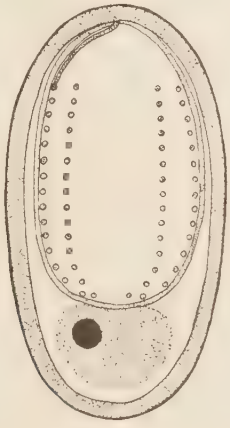


Fig. 14. A schematic representation of the longitudinal section through a mature spore of *Nosema apis*. Except for the basal portion the doubly coiled polar filament appears only in cross sections. ×10,000.

the spore of *Nosema apis* is similar to that of *Thelohania magna* (Kudo, 1920).

The fact that the polar filament of the spore of *Nosema apis* when extruded shows under certain circumstances two regions, one with a regularly large wave length, and the other with a uniformly small wave length, each having 10 to 15 turns, leads me to assume that the mode of coiling of the filament inside of the spore is a particular one. The only interpretation that can be advanced in this connection is that the polar filament of the spore of *Nosema apis* is coiled from 10 to 15 times along the polar capsule, inside of which and continuous to it, it is coiled back again toward the tip where the filament is attached. To illustrate the structure, a schematic longitudinal section of a spore is shown in figure 14.

SUMMARY

1. Among honey bees collected at Spring Valley, New York, from August 27 to September 5, 1920, 3.8 per cent. were found to be infected by *Nosema apis*.

2. Four bees harbored an undetermined Microsporidian.

3. Fresh spores of *Nosema apis* measure from 4.6 to 6.4μ long, and from 2.5 to 3.4μ broad and thick.

4. A method of applying mechanical pressure to cause the filament extrusion of microsporidian spores, is described.

5. The polar filament of the spore of *Nosema apis* is 230 to 280μ in length. The structure of the spore is similar to that of *Thelohania magna*.

6. Extruded polar filaments show two parts, one with larger and the other with smaller undulations, each composed of 10 to 15 turns, indicating that the filament is doubly coiled.

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ACANTHOCEPHALA PARASITIC IN THE DOG*

H. J. Van Cleave

There are few published records of the occurrence of Acanthocephala in dogs. The available accounts show considerable uncertainty regarding the identification of the species encountered. It seems probable that the infestation of dogs in southern Europe by *Moniliformis moniliformis* is accidental and as Porta (1914:484) has shown the case reported from Calcutta is probably that of a misidentified nematode. But one species has been recorded from dogs in North America. This species seems to have greater significance as a dog parasite than those reported elsewhere in that it seems to be a normal parasite of the dog since the worms reach full sexual development in this host.

In 1909 B. F. Kaupp described *Echinorhynchus canis* from specimens collected by J. W. Parker from a dog at San Antonio, Texas. Hall and Wigdor (1918) have recently called attention to the lack of subsequent references to Acanthocephala from dogs of this continent and have placed upon record one additional instance of an infestation by a single specimen of this species. Attempting to follow the recently proposed classification of Travassos the last mentioned authors have ascribed *E. canis* to the genus Oncicola though the explanations accompanying all of their text figures cite it as *Gigantorhynchus canis*. In their text mention is made of the difficulties encountered in attempting to determine the genus to which their single immature female specimen belongs.

J. W. Parker, the collector of the specimens upon which the original description of *Oncicola canis* (Kaupp) was based, published a note containing fairly significant additions to the biology of this species in the same volume of the journal which contained Kaupp's description. According to his account about three hundred specimens of this species were obtained from the single host individual which died of symptoms strongly indicative of rabies (Parker 1909:703). Upon post mortem "numerous ulcerations, as from abrasions three or four days old, were found on the buccal and gingival membranes and tongue;" and "about three hundred small worms (*Echinorhynchus canis*) were found in the jejunum and ileum, chiefly in the ileum, most of them attached, in some cases the head penetrating mucous and muscular coats to the peritoneum." In speaking of the possible normal host of this parasite Parker continues, "'Mad' coyotes are frequently

* Contributions from the Zoological Laboratory of the University of Illinois, No. 168.

reported in the vicinity, much more frequently than rabies is reported among domestic animals. I, therefore, think it probable that *Echinorhynchus canis* is normally a parasite of the coyote."

The writer has encountered an instance of the occurrence of *Oncicola canis* from an unusual locality. A single specimen of this species contained in the collection of Professor Henry B. Ward was taken from a dog at Lincoln, Neb., by Dr. A. D. Brewer in 1897. Reference is made to this individual by Ward (1897 : 174) as *Echinorhynchus sp?,* no attempt having been made at that time to determine the species. The present writer has examined the specimen, which has full bodily development though it is not gravid. The presence of the single female worm in the intestine of the host precluded the possibility of fertilization and embryo formation. There is no indication that Oncicola has become permanently established in the vicinity of Lincoln, for of the twenty dogs thoroughly examined in securing data for the tables published by Ward only the single instance was encountered, though "among the animals which were examined were representatives of various conditions of life under which these forms are found, both the half wild strays of the city streets and alleys and the pet animals of the home."

It is impossible to determine with certainty the source of this unusual infestation. However, since the armadillo carries the larvae of *O. canis* it seems probable that either the dog must at some time have been in the South where the larvae occur or it might have been allowed to feed upon offal from armadillos brought from the South as specimens. There is little danger that Oncicola may become established as a dog parasite beyond the geographical range of the armadillo unless it has the powers of adaptation to entirely new larval hosts. However, it seems probable that this parasite is much more prevalent as a dog parasite in the southwest than published accounts of its occurrences would indicate. Consequently since the present writer has discovered additional facts regarding this species, especially with reference to one of its larval hosts, it seems worth while to publish the results of this investigation.

Two of the original specimens of *Oncicola canis* are deposited in the Parasite Collection of the United States Public Health and Marine Hospital Service (cat. no. 9409), where they were received in October, 1902. Parker makes reference to his unsuccessful attempts to secure an identification of the specimens previous to the description given by Kaupp. The two specimens submitted to the Public Health Service fortunately include both a male and a mature female. Opportunity has been afforded the present writer to examine these specimens from the original lot and he has been able to establish the identity of these

with previously unidentified larvae from the nine-banded armadillo contained in the collections of the U. S. National Museum and of the Bureau of Animal Industry. Three lots of these larvae were collected by Dr. Albert Hassall in Texas during October, 1891, (cat. nos.: B. A. I. Parasite Collection, no. 2077; Smithsonian Institution, Hassall Collection, no. 6312 and no. 6327). A fourth lot of larvae of this species bear the date of November, 1891, and were collected by Hassall from a specimen at Washington, D. C. The demonstration of the identity of these larvae with adults of *O. canis* establishes another link in the chain of the life cycle of this species. The excessively heavy infestation of the peritoneum of the abdomen of the armadillo renders the extent of the infestation in the dog encountered by Parker readily understandable.

The absolute relation of the armadillo in the life cycle of *O. canis* is not immediataely determinable. Almost without exception an arthropod serves as the primary host which ingests the passive, hard shelled embryos of the Acanthocephala. Vertebrates which shelter the larvae of these parasites usually bear the relation of intermediate host to the parasite. Hence it seems probable that the armadillo serves *Oncicola canis* as intermediate host and that some arthropod which is used by the armadillo as food acts as primary host.

The type of this genus, *Oncicola oncicola,* is found in South America where it is a normal parasite of *Felis onca* and *F. jaguarundi.* Travassos (1917: 50) has empirically stated that the eggs of *O. oncicola* are ingested by the armadillo (Tatus sp?) and that the larvae freed in the digestive tract penetrate the wall and become encysted in the connective tissue and muscles. Until a direct infestation of the vertebrate has been actually observed it is not safe to assume that the armadillo is the primary host of either species of this genus.

Unless the specimens found by Parker (1909) represent several distinct infestations it is difficult to believe that the ulcerations of the buccal membrane "as from abrasions three or four days old" could have been caused by individuals such as I have examined from his original collection. These specimens were fully mature, the female carrying abundant, fully formed embryos. The exact time required for completion of sexual development in the definitive host is not known for many species of Acanthocephala, but in most instances it covers a period of several weeks.

The hard shelled embryos within the body cavity of the mature female vary considerably in size, ranging, for *O. canis,* from 59 to 71μ in length and from 41 to 50μ in diameter. These measurements are considerably less than those given for *O. oncicola* by Travassos (1917). According to that author the embryos of the South Amer-

ican species are 99μ long and 71 to 75μ broad. Larvae from the peritoneum of the Armadillo are usually 4 mm. or more in length.

Facts are not available to make it possible to pass final judgment upon the prediction of Parker that the coyote may be a usual definitive host of this parasite. No one has ever published a report of having found Acanthocephala in the coyote. Very little work has been done to ascertain the effect of acanthocephalan parasites upon the host. However, the experiments of Grassi and Calandruccio (1888: 524) demonstrate that Acanthocephala when present in numbers cause the host to experience great pain. Calandruccio ingested a considerable number of the larvae of *Moniliformis moniliformis*. In 19 days he was attacked by acute pains accompanied by violent ringing of the ears and of the entire head. In this instance it took five weeks for the larvae to reach sexual maturity so that eggs were recovered from the feces of the patient. It is not at all impossible that pains such as those described by Calandruccio might drive a dog or a coyote "mad."

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SOCIETY PROCEEDINGS

THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

The thirty-ninth meeting of the society was held January 9, 1920. Messrs. Boeck, Cort, Daubney, Hegner, Root, Scott and Taliaferro were elected active members. Dr. J. E. Guberlet and Dr. G. A. MacCallum were elected American corresponding members and Dr. S. Yokogawa and Dr. S. Yoshida were elected foreign corresponding members.

The following note was presented by Dr. Cobb:

NOTES ON TYLENCHUS PENETRANS AND T. DEVASTATRIX

In shipments of lily-of-the-valley bulbs from Holland, the roots show discolored areas, which on examination prove to be heavily infested with what the authorities in Holland have regarded as *T. pratensis* deMan, but which has been found to be *T. penetrans* Cobb. This pest is so common on these bulbs in Holland that the Dutch authorities have notified American authorities that they can not guarantee freedom from it. German bulbs are similarly affected.

This has raised the question as to the degree of prevalence of the parasite in this country. It has been found on roots of potatoes, cotton, violets, and camphor. Around Washington, D. C., and adjacent states, lily-of-the-valley bulbs are free from this, but harbor 19 other nemas.

In this connection it may be noted that in a shipment of about 15 species of plants from Brazil, there were found in a few ounces of attached soil 76 species of nemas belonging to 30 genera, of which species 75 per cent were new. In soil from the roots of one species of plant from Guatemala there were 23 species of nemas, of which 61 per cent were new. In bamboo from Japan, grown in South Carolina, there were eight species of nemas, of which 75 per cent were new. In dirt ballast from Trieste, Italy, 14 species of nemas were found, 79 per cent of which were new.

Red clover raising in the North-west has been seriously interfered with by the injury resulting from infestations with *Tylenchus dipsaci* Kühn (*T. devastatrix*), probably imported from Holland on hyacinth bulbs. This species also occurs on the onion. In Idaho it has done so much damage that it has been necessary to plow out second-year clover, thus occasioning considerable loss. An investigation shows that the live nemas may occur on the seed even when recleaned. As this species has been worked on by Ritzema Bos, an inquiry was sent to him in regard to the present knowledge of resuscitation of the worm. In reply he states that the eggs and larvae survive after desiccation for varying periods. In some stages the worm will survive after desiccation for 6 days; in others after desiccation for 2½ years. The worms are best revived in a well aerated film of water and may require from a number of hours to two weeks to again show signs of life. This is probably true of many soil nemas, a point of interest in connection with the wide distribution of these worms in commerce. Dr. Cobb stated his belief that the above incidents are a reliable indication of the extent to which nemas are distributed in ordinary commerce.

Dr. Cort presented the following note:

A PECULIAR MODIFICATION OF THE TAIL IN A CERCARIA

A number of theories undertake to explain the morphology of the cercaria tail. It has been surmised that the tail serves as bait for the host animal in cases where the tails resemble annelids belonging to the Tubificadae in their general outline and movement. The tail also serves in locomotion and attachment, and possibly in floating and in some instances forms a cyst for the body of the worm. In a form found in California the interpretation of the tail struc-

ture is very difficult. In the case of a similar form described by Ssinitzin, this writer has suggested that the tail serves to hold the cercaria in place in the host. An examination of the curiously modified tail in the California specimens shows that the tail appendages are connected with the excretory system and that the tail has only a weak attachment to the body, pulling loose very soon after escaping from the redia. In this species the tail could not function as a cyst, as the tail bulb has too small an aperture connecting it with the body. There are two ribbon-like processes of unknown function extending from the back, and a very long process connecting with the excretory system.

Dr. Stiles gave a very interesting talk on his experiences during the war, including helminthological work and other activities.

The fortieth meeting of the society was held February 20, 1920.

Doctor Ransom presented the following note:

INTESTINAL LESIONS IN CALVES DUE TO COOPERIA PUNCTATA

Post mortem examination of calves dying during the late autumn and early winter at the Experiment Station of the U. S. Bureau of Animal Industry at Bethesda, Maryland, disclosed the presence of heavy infestations with several species of nematodes, including lungworms, stomach worms, hookworms and a small trichostrongylid, *Cooperia punctata*. The last-named species occurs in the stomach and intestine and has been found in cattle in Europe as well as in the United States. The adult worms have been reported as occurring in the lumen of the digestive tract, but in this outbreak of disease at Bethesda it was found that they also occurred in the intestinal mucosa of the upper part of the small intestine. In the mucosa they are present in lesions which are visible to the naked eye as small accumulations of white or yellow caseous material. The same type of lesion associated with small worms, which in all probability are the same species, has been reported from calves in Mississippi and Louisiana by veterinarians in those states in verbal communications. Specimens of worms from apparently similar cases have been sent in from Venezuela by Doctor Ribero. In this material the worms are somewhat larger and more robust as a whole and in detail, the spicules attaining a maximum length of 175μ as compared with 150 to 155μ in worms collected in this country and in Europe, but the species is apparently identical. *C. punctata* is commonly associated with *Cooperia oncophora* in the intestinal lumen, the former being most numerous in the upper part of the small intestine and scarce in the lower part, the latter on the other hand being rare in the upper part and more numerous in the lower part of the small intestine. In this connection it may be noted that the young hookworms found in calves at Bethesda occurred in the lower part of the small intestine, whereas the mature worms were most numerous in the upper part of the small intestine. It is also of interest that in a number of cases hookworms were present in the fourth stomach as well as in the small intestine.

Doctor Cort exhibited a specimen of *Taenia saginata* in which one segment showed a genital papilla on each margin, the male tubules being apparently complete on both sides and the female tubules being complete and apparently functional on one side only, the other side presenting only a vaginal rudiment at the margin, which did not connect with the oviduct. The discussion of this tapeworm, a species which abounds in abnormalities, brought out other reports of similar variations and a consideration of some of the species which have been based on abnormal specimens of *T. saginata*.

Doctor Cort called attention to the fact that the Japanese government is sending 80 men each year to other countries for study and investigation, and that among these men this year there are five parasitologists, Yokogawa, Yoshida, Miyagawa, Miyauri and Goto, of whom the first two are in the United States.

Doctor Yokogawa exhibited some drawings of a Heligmosomum from the rat, *Epimys norvegicus,* in Baltimore. The species is of special interest on account of a marked asymmetry of the bursa. The discussion of this worm brought out a number of points in connection with asymmetry in nematodes and other animals, among others the asymmetry of the head in the louse, Philopterus, and of the bursa in Haemonchus and Bunostomum.

Doctor Pfender gave a discussion of oral infections and the possible relation of amebae to these infections.

Mr. Schwartz presented the following note:

ACTIVE SUBSTANCES IN MACRACANTHORHYNCHUS

Physiologically active substances that are known to occur in parasitic worms may be conveniently grouped under the headings (1) enzymes, (2) hemotoxins, (3) toxic substances which have been designated as leucomains by certain writers, but which are more commonly referred to as teniotoxins, askaron, etc., depending upon the group of worms in which they occur. Tests for the presence of enzymes in *Macracanthorhynchus hirudinaceus* (*-Gigantorhynchus hirudinaceus*) were performed on the fluid which occurs in the general cavity of the worm, on watery extracts of the entire body substance of the worm and on the fraction of the worm that is precipitated by absolute alcohol. The results were identical in each case. Neutral olive oil was not digested by the fluid of Macracanthorhynchus after a week's incubation at 37 to 38° C. Protein material such as coagulated egg albumin and fibrin stained with Congo red, showed evidence of digestion by the fluid Macracanthorhynchus by setting free the stain which colored the solution after 24 hours at 37 to 38° C. The weak proteolytic action of the fluid was observed in an alkaline medium and was completely inhibited in an acid medium. Small shreds of fibrin were not dissolved after being in contact with an alkaline solution of the fluid for a week. Proteolysis by Macracanthorhynchus fluid is therefore a markedly slow process. In contrast to the feeble digestion of proteins, digestion of starch occurred quite rapidly. Starch paste, boiled and unboiled potato starch, were converted into sugar by the fluids of the parasite. Tests for the presence of oxidizing enzymes were negative. Hydrogen peroxide remained intact after the addition of Macracanthorhynchus fluid. A tincture of guaiac was similarly unaffected, even in the presence of a weak solution of hydrogen peroxide.

Concerning the presence of hemotoxins in Macracanthorhynchus, a limited series of experiments have yielded positive results. The fluid from the general cavity of the worms was found to be hemolytic to washed red blood corpuscles of cattle and swine. Dried worm material was powdered and extracted in physiological salt solution. The opalescent supernatant fluid was tested on washed rabbit blood corpuscles with the following results. After one to two hours' incubation the corpuscles became agglutinated but not hemolyzed. Eighteen hours later, during which interval the tubes containing the mixtures of corpuscles and worm fluid were kept at 8° C., hemolysis had occurred. In the presence of normal rabbit blood serum the hemolytic potency of the extract was paralyzed. The potency of the extract was destroyed by boiling. Tests on sheep erythrocytes yielded negative results.

Doctor Cobb exhibited some apparatus useful in the collection of parasites, including (1) screens in which the intersections of the mesh are welded to make an integral construction strong enough to permit of the use of large screens, without the resultant distortion which occurs in ordinary woven mesh; and (2) a Syracuse watch glass painted black on the bottom and graduated on the bottom inside, to facilitate collection by giving a contrasting background.

The forty-first meeting of the society was held March 20, 1920.

Dr. Hall presented a note on intestinal parasites found in 18 Alaskan foxes from St. George Island; all had ascarids, ten had a very small species of

Mesocestoides, apparently new, one had an undetermined species of Taenia, a very small worm, and one had a number of dipterous larvae, probably from fly-blown flesh. None of the foxes had hookworms, which are a serious pest in some places. In comment, Dr. Cort noted the presence of a small Mesocestoides in the mouse in Colorado and a Mesocestoides from the coyote in California.

Dr. Hall also presented a short note on anthelmintics, covering two points: 1. That where oil of chenopodium is followed after an hour or longer by a purgative, Epsom salts may be superior to castor oil in that salts usually cause purgation more rapidly than does castor oil, but that where purgatives are given with the oil of chenopodium, castor oil is quite satisfactory and seems, in some cases, to have given protection of some sort apart from its purgative action; 2. That tests of anthelmintics on earthworms are only tests of toxicity for the substances tested and that the results are, for the most part, only applicable to earthworms. Tests in vitro even on parasitic worms tell little about the anthelmintic value of the drugs tested. Even the application of results obtained in administering anthelmintics to remove worms from one species of animal must be made with reserve to other species which are infested with closely related worms.

Dr. Ransom presented the following note:

THE OCCURRENCE OF ONCOCERCA IN CATTLE IN THE UNITED STATES

About ten species of Oncocerca have been reported from man and the domesticated animals. The life history of none of these has been worked out. *O. reticulata* occurs in the suspensory ligaments, flexor tendons and other connective tissues of the legs of the horse, and *O. cervicalis* in the cervical ligament of the same host. *O. volvulus* occurs in man in Africa, in nodules in the subcutaneous connective tissue usually on the body, rarely on the hand. *O. caecutiens* Brumpt, 1919, which occurs as a parasite of man on the west coast of Guatemala, is very similar to *O. volvulus,* but is located practically always on the head. It causes a disease known as coast erysipelas. Robles has operated on over a thousand cases with almost invariably prompt relief to the patient following the removal of the parasites. *O. gibsoni* occurs in cattle in Australia, usually on the brisket, and is of considerable importance in meat inspection with especial reference to the export beef trade. This species is quite certainly absent from the United States.

In the United States, either *O. reticulata* or *O. cervicalis,* and probably both, occur in the horse, and *O. lienalis* (Stiles, 1892) in the gastro-splenic ligament of cattle, the last named being common and widely distributed. There has also been found in cattle in this country, a species which occurs in a superficial position in the ligamentum nuchae and in the ligaments of the legs, especially at the knees. This form seems rather common in cattle slaughtered at Chicago. The lesions produced are slight, consisting of small local edemas and discolorations in the connective tissue and sometimes calcareous concretions in cases in which the parasites have degenerated. This parasite is of comparatively little importance in meat inspection; the species remains to be determined.

Dr. Simon reported the occurrence of an Oxyuris in the Canadian porcupine. The discussion developed the fact that this worm is probably *O. evoluta* which was described from the porcupine.

Mr. Schwartz presented the following note:

ANTIBODY PRODUCTION BY ASCARIDS

According to certain investigators, tests by the Abderhalden reaction are positive for animals known to be infested and negative for noninfested animals, but too much stress can not be laid on this fact in view of what has been

learned of this reaction. Tests by complement fixation reaction on 17 hogs, showed 16 negative and 1 positive. Fecal examinations of the same animals showed 10 uninfested and 7 infested. The positive reactor showed infestation. In this connection it should be noted that eosinophilia is infrequent in ascariasis. This fact and the failure of infested animals to show a positive reaction by complement fixation indicate that absorption of substances derived from or elaborated by ascarids is inconstant, the conditions determining the absorption being unknown. When rabbits are immunized with material from *Ascaris lumbricoides,* they show a positive complement fixation, indicating the production of antibodies. The reaction is not strictly specific, since it can be worked by antigen prepared from *Ascaris equorum.* The antigen must be kept cool and used fresh, which indicates that it is very unstable.

Dr. Ransom called attention to serum sickness occurring in his own case from the entrance of a minute amount of ascarid fluid into an abrasion on the hand. There was a prompt local reaction, rapidly extending, followed by general urticaria, slight dyspnea, pulse of 150, swelling of arms and face, and an increase of polymorphonuclears in an hour to 80 per cent.

Dr. Hassall discussed the question as to what constitutes a legitimate place of publication for scientific names. This matter is of importance since the application of the law of priority depends largely on it. The place of publication should be as legitimate as the name published.

Mr. Chapin exhibited some specimens of wasps parasitized by members of the Stylopidae (or Strepsiptera). Among these forms, the female is a permanent parasite of the wasp's abdomen. Wasps become infected as larvae, the female Stylops remaining on the wasp, while the male leaves in the spring. Wasps attack and destroy these males whenever possible and it is advisable in breeding experiments to have a partitioned cage with a screen through which the Stylops can pass and the wasp can not. The male Stylops seeks out the unfertilized female and copulates on the wasp, the female later swelling to form a bag of eggs. The resultant larvae swarm out and collect on flowers or other places where they can attach to suitable host insects. These insects carry the larval parasites to their nests, where they feed on the cell food and mature. The parasitized wasp shows pronounced changes as a result of the infestation.

Dr. Hegner presented a note on "Measurement of trypanosomes of the newt, *Diemyctylus viridescens*" (To be published in THE JOURNAL).

Dr. Scott presented the following note:

INSECTS AS POSSIBLE HOSTS OF SARCOCYSTIS TENELLA

In view of the fact that a connection had been suggested by Darling between the Cnidosporidia of the insects and the mammalian sarcocysts, a number of experiments with lambs were carried out in Wyoming to obtain information on this point. Lambs were fed numerous insects belonging to various orders and exposed in other ways to possible infection by insects. Check animals were protected from such exposure. In all cases the experiments, which were carried on for four years, gave no evidence in support of the idea that sarcosporidiosis could be associated with insects, and by raising lambs which became infected in a screen cage free from insects the hypothesis was proved untenable.

The forty-second meeting of the society was held May 8, 1920.

Dr. Cobb presented a note on "A newly discovered parasitic nematode (*Tylenchus mahogani,* n. sp.), connected with a disease of the mahogany trees" (published in THE JOURNAL).

He also discussed "The transference of Mononchs from place to place for economic purposes" (published in *Science*).

Dr. Cobb summarized a paper to be published in Nematology, dealing with the morphology of 120 new genera of free-living nematodes.

Dr. Scott presented the following note:

THE OVER-WINTERING OF THE HOUSE FLY IN WYOMING

At Laramie, Wyoming, situated at an elevation of 7,000 feet, the summers are short and the winters long and often severe. To ascertain the possibility of fly larvae or pupae over-wintering under these conditions, a feed rack in which flies were breeding abundantly in the fall was enclosed in a fly-proof cage and examined from time to time during the winter and in the spring. No flies emerged in this cage. It therefore appears that flies can not survive outdoor winter temperature in Laramie while in their larval or pupal stages. It is possible that through trains bring in flies in summer. In the discussion a general belief appeared that the warmer parts of the Southern United States probably acted as a reservoir for a fly supply during the winter and that a large number of flies must be carried North by through trains in spring and summer. Captain Daubney noted that in Mesopotamia flies disappeared during the middle of the summer, apparently owing to the intense heat. He also noted that in one case where flies became very abundant an examination developed the fact that they were breeding in enormous numbers in the horse manure which was being burned. This manure was burned on an elevated screen, after being mixed with straw, and although the manure was burning at the top, the lower layer was swarming with maggots of the so-called "camp fly" (*Musca humilis*).

Dr. Hall presented the following note:

APPARENT ATROPHY OF SPICULES ASSOCIATED WITH INCREASINGLY CLOSE AND PERMANENT UNION OF THE MALE AND FEMALE SYNGAMUS

According to the descriptions of Muehlig and Feuereisen, in *S. bronchialis* of water fowl the union of male and female is not as close or permanent in nature as in *S. trachealis;* the worms are sometimes found separated and it is possible to detach the male from the female without damaging the specimen. In the common tapeworm of poultry, *S. trachealis,* the union is more intimate and permanent; except in the case of young worms in the lungs, males and females are always found attached and it is impossible to pull the worms apart without damaging them. The bursa in this species is small and the spicules are from 60 to 140μ long; appear to be rudimentary. In the Y-worm of cattle, *S. laryngeus,* the union of the male and female is very intimate and the worms can only be detached by tearing one or the other. The bursa is very short and very thick, and the recent work of Sheather and Shilston shows that the bursa of the male is attached to the circumvulvar region of the female by the interlocking of a number of villous projections from the female inserted into crypts in the ventral surface of the bursa. No evidence of a spicule is found even in sections.

The foregoing suggests that with the development of the permanent copula there has been a strengthening of the bursal attachment and, apparently, a simultaneous atrophy of the spicules, terminating in their disappearance in *S. laryngeus* where the bursal attachment is unusually intimate. In comment, Dr. Cobb stated that an increase in the size of the bursa in species of Rhabditis is associated with a diminution in the size of the spicules.

Mr. Schwartz presented the following note:

EFFECTS OF X-RAYS ON TRICHINAE

Encysted trichinae are highly resistant to X-ray radiation. Heavy dosages exert a selective injurious action on the sex cells of the parasites. Twelve days after feeding trichinous meat which had been exposed to heavy dosages

of X-rays adult worms were found in the intestines of rats. The worms showed atrophied gonads, and in the females the receptaculum seminis contained no spermatozoa. After very heavy dosages of X-rays the worms died in the intestines of the rat without reaching sexual maturity. Attention was called to Tyzzer's experiments on the effects of radium radiation on trichinae. In discussion Dr. Cobb reported that he had tested the effects of X-rays on the gall nematode, *Herterodera radicicola.* In about a dozen tests with various exposures no effect on the nematodes was noted and the galls developed normally.

Dr. Lyon noted the effect of the X-rays in destroying spermatozoa without destroying the testicular secretions. He also called attention to the delayed production of cancer from X-rays in individuals where the cancer developed years after exposures.

The forty-third meeting of the society was held May 29, 1920. Dr. Stiles presented the following note:

RECENT INVESTIGATIONS ON EXCRETA DISPOSAL

The factors involved are those of expense and labor. With this in mind, the Nasik method was tested, this being in use in parts of India. A trench is used and the excreta covered with street sweepings; it has been claimed that these trenches are inoffensive and do not breed flies, and that the excreta are available as manure in a year. The availability of sawdust led to testing this and it was found excellent. Since burying excreta brought them closer to the ground water and removed the surface soil with its bacteria, the excreta were left on the ground surrounded by a box-like container of sawdust and were covered with sawdust. There was no odor, the sawdust would not wash away or blow away and would not burn. This method is only applicable where there is plenty of sawdust, but this is the case in the pine woods region through a large part of the rural section where present conditions are insanitary. It dries on top and hookworm larvae can not come to the surface through it; they probably die in a year. Amebae may live up to about 52 days, possibly longer. Allowing a 4-inch range of capillarity, about 6 inches of sawdust will be sufficient cover. Flies will develop from eggs and larvae in the manure and various birds will eat large numbers of the flies as they emerge. The fly problem is not entirely solved as yet.

Tests with wells and pits show that the transport of bacteria through soil is a question of ground water. Bacteria do not travel through thoroughly dry soil; they apparently travel large distances very rapidly with ground water. Contrary to what has been stated, ground water contains ciliates and flagellates.

Capt. Daubney presented a note on the lungworms, *Dictyocaulus filaria* and *D. viviparus,* reviewing his own work on the life history. Artificial infection of experimental animals was accomplished first by Romanovitch and Slavine and later by Guberlet. Daubney also succeeded in infecting sheep with *D. filaria* and exhibited figures illustrating the stages in the life history. He called attention to the confusion of the larvae of Dictyocaulus with those of Synthetocaulus by some writers. He also noted that the larvae are not as resistant to actual desiccation as some have claimed, and where there is a trace of moisture worms may survive for much longer periods. The larvae ascend a moist surface in dampness or diffused light and descend when exposed to direct sunlight.

Capt. Daubney also read a summary of a study of the lesions due to various species of lungworms, noting that the lesions were different in the case of Synthetocaulus from those in the case of Dictyocaulus.

Mr. Chapin reported the occurrence of encysted *Gongylonema scutatum* in *Aphodius rubeolus,* a new host for the larvae of this worm.

Dr. Scott presented a note in regard to the transmission of swamp fever by means of the nasal secretions. The secretions were taken from infected horses and injected subcutaneously or injected into the nostrils. In both cases the experiment animals developed the disease, thereby demonstrating this method of transfer for the first time. It is possible that insects may carry these secretions from the nostrils of one animal to another.

The forty-fourth meeting was a dinner celebrating the decennium of the Society.

MAURICE C. HALL, Secretary.

NEW HUMAN PARASITES

Necator argentinus Paroli, 1920, is described from Argentine and southern Brazil (Sem. med., Buenos Aires, no. 6, 1920). Langeron (Bull. soc. path. exot., 13:539) discusses the significance of the cervical papillae in the Ancylostomes and shows that they are not of diagnostic value. He concludes that the Argentine species is probably the same as *Necator americanus* Stiles.

Entamoeba macrohyalina Tibaldi, 1920, was found in the crypts of the tonsils of two young persons in Italy. It measures 24 to 40μ in diameter with a nucleus measuring 3 to 6μ in diameter, and thus tends to be considerably larger than *E. gingivalis* which is of common occurrence in the mouth and may also be found in the tonsils. It differs conspicuously from *E. gingivalis* in the character of its ectoplasm which forms a broad homogeneous zone of a pale opal tint when stained with Giemsa. There is a well developed contractile vacuole evident in the living organism. The nucleus is without a definite membrane and there is no karyosome. The nuclear chromatin abundant, and in the resting condition is disposed in little clumps closely pressed together in the peripheral portion of the nucleus, leaving in the center a small clear triangular or polyhedral area. (Ann. d'Igiene, 30:613-620, 1 pl., figs. 1-12, Oct., 1920.)

BOOK REVIEWS

Die Tierischen Parasiten des Menschen, die von ihnen hervorgerufenen Erkrankungen und ihre Heilung. By Max Braun and Otto Seifert. II Teil: Klinik und Therapie der Tierschen Parasiten des Menschen. By Doctor Otto Seifert. Pp. 506. Leipzig: Verlag von Curt Kabitzsch, 1920.

The first part of this well-known text appeared just before the opening of the war and was reviewed at length in the Journal (2: 201). The second part, covering the clinical-therapeutic section, has only just appeared. The delay permitted the complete reorganization of the text and the introduction of material from the period of the war so that the book contains much not yet available in any other text. There is, of course, no necessary and intimate connection between this part and the section that appeared earlier under the immediate authorship of Professor Braun, but by virtue of a common origin and general title one might expect an agreement in formal matters at least. The fact is, however, that the names employed in this part to designate the parasites are noticeably different from those employed by Braun, even in some cases being conspicuously unusual, antiquated, and erroneous. This is unfortunately calculated to confuse most students and will surely lead astray those without considerable technical knowledge in this exact field.

The author handles his topics in a fashion not found in any other work, emphasizing symptomatology, treatment, drugs, dosage and other practical medical aspects that are peculiarly significant for the practicing physician and make the volume indispensable. Reference to the sources from which material is taken are introduced in such abundance that fifteen to twenty-five percent of every page is taken up by the brief footnotes containing the references. The bulk of the material is German of course and American cases are not very fully, or carefully, listed; but other nations are equally passed over and perhaps the cases listed, which are evidently selected, give adequate illustrations of types as well as methods of treatment and results. The author cites often a review or abstract of an article rather than the original, his references being confined to relatively few journals. This has the advantage of making it possible to consult the article readily but is open to some objection by virtue of greater chance of error and confusion. The great medical journals in Germany and England are most often cited; those of France and the United States come next and all others are infrequently referred to. Thus while citations are abundant and representative, the treatment of the topics is not exhaustive though more extensive than in any other work yet published.

The arrangement of the material seems at times somewhat peculiar and difficult to explain. Thus in the general section on Cestodes many pages are taken up by a discussion of hookworm anemia, of ascaris symptoms and toxins, of whipworm anemia, of pinworm poisons, and of verminous appendicitis. This material is of special value and deserves greater emphasis. Since no general discussion appears under the heading Nematodes, it is possible that this material really represents a misplaced section which by chance was printed under the wrong heading. A parallel case occurs under the heading Myiasis externa where at the close several pages are devoted to organisms surely not in any sense flies, such as the earworm, various Myriapods, Artemisia, and even a Milleporidian coral that produces dermatitis.

The volume has a good author's index but the topical index is weak, as important a parasite as the trichina not being referred under any heading. But these are minor defects and do not conceal the high value of the work. In its first edition Professor Seifert's section represented a new departure in the literature of parasitology. In this new edition the book is larger, better, and even more indispensable than before.

PARASITES AND PARASITOSIS OF THE DOMESTIC ANIMALS. The Zoology and Control of the Animal Parasites and the Pathogenesis and Treatment of Parasitic Diseases. By B. M. Underhill. Cloth. Pp. 379. New York: The Macmillan Company, 1920.

In this text the author presents the subject from the standpoint of a veterinarian. He devotes two chapters to general questions concerning parasitism, eleven to arthropods, twelve to worms, and two to protozoan parasites. This represents perhaps the needs of veterinary practice but is inadequate surely to portray present knowledge of the field.

As the only presentation of the subject in English from a veterinarian, the work is valuable; it indicates what topics appear important in veterinary science, and what aspects of those topics deserve emphasis. The book also contains much that is not found in any recent treatise on parasitology and thereby will command for itself a place in the literature of the subject. It is unfortunate that it displays in some respects a lack of finish that detracts greatly from the effect. The paper is so thick that the book appears padded, the typography is not at all attractive and some pages are very poorly set. Are we to attribute to the war these shortcomings on the part of publishers who ordinarily set and maintain high standards?

Those figures which are copied from the United States Bureau of Entomology are mostly admirable but a few (p. 61) are unnecessarily large and coarse. Some other figures (p. 158) are so poorly copied as to be a reflection on the source and among the original drawings too many are rough and unattractive or even mere caricatures. This fault, which is marked in many recent works, obtrudes itself on the attention and prevents the excellencies of the text from receiving due acclaim.

NOTES

The International Health Board of the Rockefeller Foundation has recently approved a plan for a cooperative investigation on the biology of hookworm larvae in the soil to be carried out by the Department of Zoology of the School of Hygiene and Public Health of the Johns Hopkins University. The investigations will be carried on in Trinidad in connection with the International Health Board's hookworm campaign which is under the direction of Dr. G. C. Payne. The expedition will start about May 1st and will be gone about four months. It will be under the direction of Dr. W. W. Cort of Johns Hopkins University, and associated with him Dr. J. E. Ackert of Kansas Agricultural College and Mr. D. L. Augustine. It is planned to center the researches around the question of the infectivity of the soil and to study the various phases of the life of the hookworm larvae in the soil which relate to this problem.

The Typhus Research Commission of the League of Red Cross Societies to Poland has printed a very important preliminary report of their work (Int. Jour. Pub. Health, 1:211). They found the *Rickettsia prowazeki* of da Rocha Lima constantly in lice fed on typhus patients and also in the vascular lesions of experimental animals infected with typhus. These lesions are thoroughly characteristic of the disease.

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